# BIOLOGICAL BULLETIN

OF THE

Woods Hole, Mass.

## Marine Biological Laboratory

WOODS HOLE, MASS.


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VOLUME XXX.

WOODS HOLE, MASS.

JANUARY TO JUNE 1916

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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OF THE

**Marine Biological Laboratory**

WOODS HOLE, MASS.

VOL. XXX JANUARY, 1916 No. 1

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PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
*28 Essex Street, Strand*
*London, W. C.*

AGENT FOR GERMANY
R. FRIEDLÄNDER & SOHN
*Berlin N. W.*
*Carlstrasse, 11*

**Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00**

Entered October 10, 1902, at Lancaster, Pa., as second-class matter, under Act of Congress of July 16, 1894.

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All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa.

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# BIOLOGICAL BULLETIN

## CAUSES OF STERILITY IN THE MULE.[1]

J. E. WODSEDALEK.

(NINE PLATES.)

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### INTRODUCTION.

For several years the author has been contemplating to make a study of the germ cells of the mule with the aim of offering a thorough explanation of the causes of sterility in this hybrid, but it was not until the past year that material for such an investigation was available. Cytological studies of the sex cells, especially those of animals, in recent years have added much to our knowledge of the mechanism of heredity; and such studies have kept fairly good pace with the comparatively rapid advances in our knowledge of plant and animal breeding which have been made since the rediscovery in 1900 of the results of Mendel. And while this study is now recognized as one of the chief methods of confronting many problems in genetics, and must be carried

[1] From the zoölogical laboratories, University of Idaho.

on in correlation with animal and plant breeding before any adequate explanation of the results of hybridization can be obtained, the study of the germ cells in hybrids themselves has thus far been somewhat neglected.

Guyer ('00) in his excellent work on the spermatogenesis of normal and hybrid pigeons says: "It is a remarkable fact that no attempt has been made so far to investigate carefully the spermatogenesis or ovogenesis of hybrid forms. In all the mass of literature discussing or touching upon hybridism, so far as I have been able to ascertain, there has been in no instance an approach to a thorough study of the germ cells. Yet almost every writer states that through the study of hybrids, we have perhaps the best opportunity for gaining a clew to many of the most vital points in the great problem of heredity. A number of investigators have remarked that in certain instances the anthers, ovary, or testes as the case might be, were defective, and have let the matter go at that."

It is even more remarkable now, since fifteen years have elapsed and no further attempt has been made to investigate carefully the spermatogenesis or ovogenesis of other hybrid forms. Professor Guyer's work on hybrid pigeons which was published in 1900, the same year in which Mendel's investigations were rediscovered, so far remains as the only outstanding piece of work on that particular subject. It is all the more remarkable when we consider the fact that such a great deal of cytological work has been done during the past fifteen years and that by far the largest bulk of the work on hybridization has been done during the same period.

The mule is probably one of the best known hybrids for he is raised in practically all parts of the civilized world. He has been known for many centuries and has been used more or less in Europe since the days before Christ, for Varro who wrote in the first century B.C. refers to mules in Roman agriculture. Sterility in mules has no doubt been a subject of discussion for as many centuries but the real nature of the cause of sterility in these hybrids has heretofore never been carefully investigated. The spermatogenesis of the horse which was carefully worked out at the University of Wisconsin Zoölogical Laboratories a

year ago (Wodsedalek, '14), has rendered several phases of this difficult problem much more intelligible than would have been possible otherwise. It might be added that certain phases of this problem would have become even more perspicuous had the spermatogenesis of the ass also been carefully worked out and thoroughly understood; but unfortunately the jack material has so far been unavailable.

## Comparison of the Horse and the Ass.

Hayes ('04) says: "Owing to the extreme want of uniformity in the gaps left, during the process of evolution, between descendants from similar ancestors, we are unable to lay down any exact general rules for classification. The inclusion of horses, asses, and zebras in the genus *Equus* admits of no controversy, because they are the only possessors of the distinguishing characteristic of having only one complete hoofed toe on each foot."

While there seems to be no doubt that the horse, ass, and zebra have descended from common ancestors, at the present time, there is considerable difference between the horse and the other two Equidæ. The only distinctive difference between the asses and zebras in general seems to lie in the tiger-like stripes which are invariably lacking in the ass. The differences between asses and horses are marked, as can be seen from the following quotation of a detailed comparison by Hayes ('04). "Some of the following differences between asses and horses are relative, and others absolute. Most of these differences also exist between zebras and horses.

"1. The ass has, practically speaking, chestnuts only on the forelegs, which is a peculiarity that is met with in certain breeds of horses (p. 319). In some cases, the ass has vestiges of chestnuts on his hind legs. The chestnuts and ergots (p. 319) of the ass are much thinner than those of the horse.

"2. The ass has a tufted tail, somewhat like that of an ox, an erect mane, and no forelock. The horse, with the exception of Prjevalsky wild horse (p. 640), has a bushy tail, drooping mane, and a forelock, when they have been allowed to grow. The difference in the mane is due to the length of the hairs of the part. In almost all breeds of horses the hairs of the tail grow long from

the root of the dock. In the ass they do so only as they approach the end of the dock.

"3. As a rule the ass has five loin vertebræ, and the horse six (p. 422). I have never heard of an instance, in the domestic ass, of the number of these bones exceeding five. If, however, we examine the skeleton of the Mountain Zebra which is in the museum of the R. C. S. Lincoln's Inn Fields, we shall see that it has six loin vertebræ. The number of these bones is subject to variation in all vertebrates.

"4. In the horse, the lachrymal duct, which is the canal that conveys tears from the eye on each respective side into the nostril, has its opening near the inferior commissure of the nostril, and on the line of union between the dark-colored skin and the pink mucous membrane. In the ass and mule, it is situated at the inner face of the outer wing of the nostril. This orifice is sometimes double.

"5. In the ass, the false nostril extends higher up than in the horse.

"6. The male ass has two rudimental teats in the form of small tubercles. They are usually absent in the horse.

"7. The vocal sounds of the ass (braying) are produced in a different manner from those of the horse (neighing). . . . We may, therefore, conclude that braying can be performed only during strong contractions of the muscles of the abdomen and chest. It is evident that this muscular contraction is not required in the neighing of the horse.

"8. In the ass, the deep depression at the base of the epiglottis is covered by a thin membrane, which is capable of vibrating, and which is wanting in the horse. It may have some influence in causing the voice of the ass to differ from that of the horse. . . .

"9. The ass hardly ever has any irregular markings on its coat such as a 'star,' 'blaze,' 'reach,' or 'stockings,' all of which are very frequent among horses. A small star, on one or two occasions, is the only mark of the kind I have ever seen in the ass, of which animal I have not had much experience.

"10. I believe I am correct in saying that the color of the ass is never of a bright bay, chestnut, red or blue roan, or nutmeg gray. I have seen mules of an iron-gray color; but have not

observed it in the ass. This conservatism in color and freedom from irregular markings, shown by the ass, is very remarkable; considering how greatly the coat of the horse varies in this respect and that the ass has, in all probability, been longer under the influence of domestication than the horse.

"11. The ass is higher over the croup, than at the withers which is a peculiarity that tends to make his withers appear unduly low (p. 241). The spines of the vertebræ at the withers are only a little shorter in the ass than they are in the horse. As a rule horses are higher at the withers than they are at the croup.

"12. The horse's dock is thicker, stronger, and shorter than that of the ass.

"13. The horse, on each side of his croup and covering his pelvis, has, underneath, and closely adhering to the skin of the part, a thick and extremely dense layer of connective tissue, which is so close and hard, that it looks like horn, when the skin has been tanned and dried. These two patches of thickened skin are separated from each other about four or five inches apart, so that there is a strip of skin of ordinary thickness running down the croup towards the tail. . . . The 'shell' is connected to the skin so closely that the two form one piece; although their respective consistencies are different. If a section be made through the hide their line of union may be readily seen. In the ass, the 'shell' is not confined to the skin that covers the pelvis; but also extends over the ribs, which are consequently not as sensitive to the effects of blows as are those of the horse. . . .

"14. The ass has no tufts of hair at the fetlocks (p. 290).

"15. Messrs. Tegetmeier and Sutherland appear to have been the first to note the difference between the respective periods of gestation of asses and horses; the former period being twelve months; the latter, eleven months.

"16. The foot of the horse is more highly specialized than that of the ass. One of the best marked evolutionary changes in the equine foot, was the gradual curtailment of its posterior bearing surface (frog and sole), until, in the horse (Fig. 382), the length of this bearing surface is not much greater than its width, and is included between the heels and the 'toe' of the hoof. In the ass, this bearing surface is relatively longer than in the horse,

and extends some distance beyond the heels to the rear (Fig. 443). Also, the shape of the foot of the ass, from above downwards, is more or less cylindrical; and that of the horse is more or less in the form of a truncated cone.

"17. The teeth of the horse are more highly specialized than those of the ass. . . . We can see in Fig. 643, that the gradual lengthening of the crowns of the molar teeth is a well-marked feature in equine evolution; and from a study of the mechanism of equine dentition, we learn, that for purposes of food-prehension and mastication, the growth of the incisors must be proportionate to that of the molars. Hence, we may assume that the crown of the incisors, like those of the molars, have gradually lengthened during the evolution of the horse; and consequently that the acuteness of the angle made by the upper and lower incisors of the horse of today increases with age, to a greater extent than it did in the case of his ancestors. On turning to the domestic ass, we find that in old donkeys (Figs. 448, 449 and 450), the angle in question is greater in the horses of similar ages (Figs. 445, 446, and 447). Consequently we may infer that the teeth of the domestic ass are of an older type than those of the horse. Also, the incisors of the donkey are relatively narrower than those of the horse." In addition it might be said that the ears of the ass are much larger and longer.

All of the differences enumerated above are skin-deep, or anatomical in nature. The present study has revealed another difference, a cytological one, which is probably the most important, for it lies in the finer structure of the cells of these animals and no doubt is at the base of all of the differences perceptible to the naked eye. It was learned that the cells of the horse contain thirty-seven chromosomes (Wodsedalek, '14). The present study shows that the cells of the mule possess fifty-one chromosomes. This would suggest that the cells of the ass possess about sixty-five chromosomes, if nothing unusual happens in the development of the hybrid, thus making the remarkable difference of twenty-eight chromosomes between the cells of the horse and those of the ass. This matter is considered at length in the latter part of the paper.

## CHARACTERISTICS OF THE MULE.

The mule is a hybrid having for sire a jackass or male ass, commonly termed a jack, and a mare for dam. If, however, a stallion be bred to a she-ass which in the United States is called a "jennet" and in England a jenny, the result is a hybrid known as a hinny. It seems to be a well-established fact that no special distinction with respect to appearance or conformation can be made between mules and hinnies, because in both of these hybrids of both sexes the proportional resemblance to the horse and ass is of infinite variety. Mares are generally taller than jennets, hence the mules as a rule are of a greater height than hinnies, which appears to be the only difference between these two hybrids.

There seems to be a great deal of difference of opinion as to which one of its parents the mule most resembles. For example, Hayes ('04) says: "The coat, mane, tail, feet, and voice are more or less intermediate between those of the horse and those of the ass. The hind chestnuts in some cases are well developed, as in the ordinary horse; in others, they are in a comparatively vestigial form, and one or both may be absent. These hybrids "resemble the ass more than the horse in their placid temper, great adaptability to work, and longevity. In carrying or drawing loads they are superior to horses of the same weight, and they can obtain the necessary energy from food which horses cannot digest, as for instance, the reeds on which the mules of the south of France live. I have experimentally established the fact that their digestive power is higher than that of horses" (Sanson).

Lydekker ('12) says; "In the case of both mules and hinnies the general build and appearance of the animal accord with the type of the sire, although in the manner of bodily size the dam is followed. Mules are therefore asinine in appearance, although with a more horse-like tail, and relatively large ears; whereas, the more horse-like hinny is small. If, however, females of the great Poitou ass were to be utilized for hinny-breeding, the progeny would probably be of larger stature. One exception to the ass-like character of the mule is that it lacks the white belly of its male parent."

Wilcox ('07) says: "It is not true, however, as sometimes asserted, that the mule eats less than the horse. On the contrary

the mule has an excellent appetite. However, the mule can go longer without food and can live on coarser and more unpalatable food than could be expected to give results with the horse. Contrary to a prevailing belief also, the mule is equally susceptible to various diseases as the horse. In the Philippines our mules suffered as much as horses from surra. Glanders has always been dreaded as one of the scourges of the army mule. Even colic and other digestive troubles familiar to horse raisers also occur among mules."

Plumb ('06) says: "The characteristics of the mule partake of both sire and dam. There is the long ear, slender body, tufted or slightly haired tail, and small, slender foot, and braying voice of the ass."

"The temperament of the mule is quiet and patient, while for steadiness under the collar and hard pulling he has no equal in the equine world. . . . Horses are more nervous and uncertain in temperament than mules, and are more subject to fright and consequent runaway.

"The endurance of the mule is remarkable. . . . Mules usually live to a greater age than horses, and perform their work with regularity and on less feed, a most important point in their favor. Cases are recorded of mules living to seventy years of age. . . . The resistance of the mule to disease, its activity, sureness of foot, docility, and easiness of keep, have resulted in its finding much favor in the army service." The sureness of foot is no doubt inherited from the ass.

That mules and hinnies are sterile is a well-known fact. And if degeneration of the sex cells of all of these hybrids is as pronounced as it was found to be in the material studied in this investigation—and in all probability it is—the cause of sterility will remain obvious. A few cases of fertile mules have been recorded, but careful investigations show that such records are not authentic and practically all of the writers on mules regard them as unreliable.

Lydekker ('12) says: "With the possible exception of a few instances in which the female is stated to have produced offspring, the mule is sterile; as, indeed, might be expected to be the case when the difference between its parents is borne in mind."

Hayes ('04) in remarking about the matter of sterility of mules says: "Scientific research has amply proved that mules and hinnies are absolutely sterile, although cases are on record of induced lactation occurring in females of this kind. . . . Accounts not infrequently appear in the American and other papers, of mules which are seen suckling young, and the conclusion is at once arrived at that these young are the offspring of the animals that are supporting them, but it may be regarded as perfectly certain that they are merely adopted foals, which by their endeavors to suck female mules have developed in the latter abnormal lactation."

## Material and Methods.

The material for this study was obtained through the courtesy of my friend, Dr. J. W. Kalkus, professor of histology and pathology at the State College of Washington, Pullman. Dr. Kalkus made every possible effort to secure the material for my studies and generously placed at my disposal material obtained from three animals.

The hybrids were about two years old at the time the material was removed from them. It is probable that all three of the mules were by the same sire. The tissue was fixed in Flemming's fluid, and stained with Heidenhain's iron hematoxylin. The sections were cut from five to nine microns thick.

## Appearance of the Testicular Tissue.

The first noticeable difference in the testicular structure of the mule and the horse is that the seminiferous tubules of the mule are in general much smaller than those of the horse. There is considerable variation among the tubules of the mule. The diameter of some tubules is fully twice that of others, and all gradations between the two extremes may be found. The large tubules are characterized by large clear areas in their lumens. This clear space diminishes as a rule in proportion to the diameter of the tubules. In most of the small tubules the cells occupy all of the lumen. This structure, however, is not always the case in the respective tubules, for occasionally we find large tubules with solid lumens and on the other hand small tubules with clear lumens.

The difference in the interstitial cells is quite obvious. They are much larger, much more numerous, and more regularly distributed in the horse than they are in the hybrid. The nuclei in the former are large and usually contain one or two large nucleoli, and several small karyosomes; while those of the mule are small and contain many small karyosomes, and the large nucleolus is not as constant as it is in the horse. The cytoplasmic content of these cells in the horse, too, is larger and not as variable in size as in the mule.

### Spermatogonia.

The spermatogonial cells of the mule are considerably larger than those of the horse in the corresponding stages. They generally occupy the usual position next to the tubule wall, though occasionally they are crowded out of this position by the numerous nurse cells and pushed further in toward the lumen. They occur in relatively fewer numbers in the hybrid than they do in the horse. In cross sections of the tubules they very seldom form a complete ring within the tubule wall, and are never found in a crowded condition neither along the entire tubule wall nor in small areas, as is often the case in normal mammalian tissue. The cells are usually far apart, and it seems safe to state that in a large majority of the tubules only about twenty to thirty per cent. of their inner surfaces is covered with these cells. It seems equally as correct to say that in cases where the spermatogonial cells are most abundant they never exceed covering more than sixty per cent. of the tubule's inner surface; and it might be said that such extreme cases are rarely found. Many of the tubules contain only a comparatively few spermatogonial cells and not infrequently tubules are found in which no cells can be identified as such. The cells occupying the position usually occupied by the spermatogonia greatly resemble the ordinary nurse cells.

During the prophase of the spermatogonial cell the nucleus is round and contains a large nucleolus which is usually heart-shaped, several small karyosomes, a large number of small granular masses and extremely thin linen strands (Figs. 1 and 2). In this respect the cells resemble those of the horse in the corresponding stages, except that they are much larger. No centrosome can be detected at this stage although a dense mass of

cytoplasm can often be seen near the nucleus. This is in all probability the idiozome. These cells have definite walls which mark them off clearly from the neighboring cells. They are almost spherical and have the appearance of smear cells, a condition presumably due to the fact that they are not crowded.

As growth proceeds all parts of the cell increase in size. When the maximum size is reached, dense masses of chromatin make their appearance which become more and more defined until the chromosomes of various shapes and sizes are formed. Sometimes the chromosomes are so close together that an accurate count is impossible; but more frequently they are sufficiently separated so that many definite counts were possible.

There are fifty-one chromosomes found in the spermatogonia of the mule. Fifty of these are the ordinary chromosomes or autosomes, and one is the accessory which is larger than the others. Fifty-one chromosomes is fourteen in excess of the number found in the spermatogonial cells of the horse which has, at least in some breeds, a total of thirty-seven, thirty-six autosomes and one accessory (Wodsedalek, '14).

A considerable amount of time was spent in an attempt to ascertain which of the chromosomes are of paternal and which of maternal origin, or in other words which were contributed by the jack and mare respectively. And while the author has obtained some fairly definite ideas in regard to the individuality of the chromosomes in the mule, he does not feel that any positive statements in regard to the matter can be made until the sex cells of the ass will have been thoroughly investigated.

It is quite certain, however, that the accessory chromosome retains its individuality, for its appearance in the mule is identical to that of the accessory of the horse. The mule no doubt received the accessory from the mare in the same manner as it is contributed by the dam to her male offspring in the horse, in view of our knowledge of the behavior of this body in the spermatogenesis of the horse (Wodsedalek, '14), and particularly according to our acknowledge of the behavior of the accessory chromosomes in relation to sex determination in another mammal, the pig (Wodsedalek, '13). In regard to the other chromosomes it is definitely known that in the horse they vary considerably

in size and shape (Wodsedalek, '14), and the same is probably the case among the chromosomes of the ass. And the fact that some of the chromosomes of the ass are apparently of the same size or about the same size as some of the chromosomes of the horse, makes it rather difficult to discriminate all of the maternal from the paternal chromosomes in the hybrid. It appears, however, that most of the large chromosomes in the mule are paternal in origin. A careful study of the spermatogenesis of the ass should throw considerable light on this subject.

It is also definitely known, as was stated before, that the horse, at least of some breed or breeds, possesses thirty-six chromosomes besides the accessory, or sex-determining chromosome, in the spermatogonial cells. The reduced number of the ordinary chromosomes in this animal is eighteen, which number enter into the formation of the one type of sperm, and eighteen plus the accessory or nineteen in the other type. Since the spermatogenesis of the horse with regard to the accessory (Wodsedalek, '14) bears such a striking resemblance to the spermatogenesis of the pig, in which animal the problem of sex-determination was carefully worked out (Wodsedalek, '13), the author in his paper on the spermatogenesis of the horse assumed that the sperm cells possessing eighteen chromosomes are male determining and those possessing nineteen are female determining. It was further assumed on account of the behavior of the accessory in the horse that the oogonia of the dam contain thirty-eight chromosomes, or thirty-six autosomes and two accessories, while the mature ova contain the reduced number of nineteen chromosomes, or eighteen autosomes plus one accessory.

The above figures are in all probability correct, but the actual count of the chromosomes in the female tissue must be made before it can be regarded as authentic, regardless of the strong evidence obtained in favor of the assumption through the comparison of the behavior of the chromosomes in both the male and female germinal and somatic cells of the pig. Great as the similarity is, the mere fact that the pig is the only case among the vertebrates in which the relation of the accessory chromosomes to sex-determination has been clearly and completely demonstrated, any remarks based on such a comparison must necessarily be of a more or less reserved nature.

The investigations on the relation of the accessory chromosome, or group of chromosomes, to sex-determination in at least another vertebrate, the man, must not be overlooked. And while there is some difference of opinion in regard to this matter in man and the results are not entirely conclusive, they should nevertheless add more strength to the above assumption. Guyer ('10) found that the spermatogonia of man contain twenty-four chromosomes; and that twelve chromosomes appear for division in the primary spermatocyte of which ten are evidently bivalent and two accessories. During division ten chromosomes pass to one pole and ten plus the two accessories to the other, giving rise to two different types of secondary spermatocytes which eventually give rise to two types of spermatozoa; the one type containing ten chromosomes and the other ten plus the two accessories.

Montgomery ('12) confirms the number of chromosomes found by Guyer but disagrees with him in regard to the accessory. Von Winiwarter found forty-seven chromosomes in the male, of which forty-six unite at reduction and form twenty-three bivalents and the accessory remains unpaired. Two types of spermatozoa are produced, the one containing twenty-three chromosomes and the other twenty-three plus the accessory, or twenty-four chromosomes. In the female he had some difficulty in obtaining the exact number of chromosomes, but his best counts gave forty-eight, which number fits in with the results obtained in the male.

The cause of the difference of opinion is probably due to the fact that Guyer used Negro material in his investigation, while Von Winiwarter studied tissue obtained from a white man. In view of the difference between these two human races it is not at all improbable that a difference in the number of chromosomes also exists. The Negro is fully as far removed from the white man as is the ass from the horse, where a great difference in the number of chromosomes apparently exists. This vast difference in number appears to be, at least in part, responsible for the sterility of the mule. The difference between the white man and the Negro in regard to the number of chromosomes according to Guyer and Von Winiwarter is equally as great, but unfortunately the mulatto is fertile.

The number of chromosomes in the spermatogonial metaphase stage of the mule is, as was stated before, fifty-one, or fifty autosomes and one accessory. The number in the corresponding stage of the horse is thirty-seven or thirty-six autosomes and one accessory; while the reduced number is eighteen in the one type of sperm and nineteen, including the accessory, in the other. According to the previous discussion it seems fairly safe to predict that in the oögonial cells of the mare there are thirty-eight chromosomes or thirty-six ordinary chromosomes and two accessories, and that the reduced number in the matura ova is nineteen including the one accessory. This would suggest that nineteen of the fifty-one chromosomes including the accessory are maternal in origin or contributed by the dam, and the remainder or thirty-two are paternal in origin or contributed by the jack. On the customary inference that the reduced number of chromosomes is always one half the number in the spermatogonial and somatic cells we should expect to find sixty-four chromosomes in the ass, with the possible addition of one accessory in the jack and two in the jennet; thus making a total of sixty-five and sixty-six respectively in the two sexes of this animal.

These plausible figures for the jack and jennet would, of course, be expected only in the event that no cellular abnormalities occurred in the mule from the time of the fertilization of the dam's ovum by the jack's sperm, through the succeeding developmental stages of the hybrid up to maturity. In such a case the full quota of chromosomes would be handed on to the spermatogonial cells of the mule.

The normal mitotic figures of the spermatogonial cells seem to indicate that probably no such abnormalities occur in the various preceding stages. But even such an apparently normal condition cannot be relied upon too strongly. For while the spermatogonial cells are normal during mitosis, as near as can be detected, that does not necessarily preclude that nothing unusual happens in the developmental stages of the hybrid, particularly at the time of fertilization and early cleavage stages. At any rate it would not be at all surprising if something unusual did happen to bring about the fifty-one chromosomes. The need of a careful study of the chromosomes in the jack is obvious. A

study of the sex cells in both sexes of the hinney, as well as those of the female mule and she-ass or jennet, would also add materially to this immensely interesting problem of sterility of the horse and ass hybrids.

Fig. 3 represents a polar view of the metaphase stage of a spermatogonial cell. The fifty-one chromosomes are distributed throughout the entire plane of the equator and the larger ones, as a rule, are arranged along the edge. The heart-shaped accessory can be readily distinguished not only on account of its shape, but also on account of its large size. It is invariably at or near the edge of the equator. Fig. 4 is a side view of the spindle and a representation of one of the many perfect spindles found in this stage. There are no scattered chromosomes to be seen; each enters the equatorial plate and divides. The fact is further evidenced by the perfect divisions which follow. During the anaphase stage the chromosomes move to the opposite poles. Figs. 5 and 6 show that there are no stragglers among the chromosomes. The accessory which also divides in this stage, can practically in all cases, be detected on account of its larger size. In the telophase stage when the chromosomes are at the opposite poles the cytoplasm begins to constrict in the center. Soon the chromosomes become ragged and later disintegrate while the nuclear membrane makes its appearance. Following the last spermatogonial division the cell enters into the period of great growth and represents the early stage of the primary spermatocyte.

## Primary Spermatocytes.

### 1. *Early Prophase.*

The most important phase of this problem lies in the primary spermatocytes. These cells are the result of the last spermatogonial division as in normal forms, and while their behavior is always interesting and more or less difficult to analyze in any case of spermatogenesis, it is especially so in this hybrid. From the time of the appearance of these cells until the period of their final destruction one is confronted with numerous fascinating categories.

During the early prophase the primary spermatocytes resemble

the early spermatogonial stages, except that the cells are larger and the linin strands a little more conspicuous. The large nucleolus is again very distinct and appears as it did in the spermatogonia. A large, irregularly shaped karyosome is also usually present, as are a number of small ones. As the cell increases in size the linin strands become more conspicuous and the chromatin granules more numerous. The karyosomes, too, become larger and are surrounded by dense masses of chromatin granules. One of the karyosomes is always large and has from eight to twelve linin strands radiating from it. Frequently a few strands can be seen radiating from the small karyosomes. These radiating strands together with many others, form a continuous network of linin. Later the chromatin granules begin to mass around the linin strands and the karyosome begin to disappear (Fig. 7). Soon all of the chromatin material seems to be arranged along the linin strands, which take on the appearance of a network of chromatin threads. The threads are very granular and vary a great deal in length and somewhat in thickness (Fig. 7).

There is no definite arrangement of these threads in the network of the various cells. Each cell seems to have its own hit-and-miss tangle. In some cells the long threads are more numerous and the short connecting threads are proportionately fewer in number. In others there are many short components forming the network. The nuclei at this stage vary considerably in size.

Synizesis, which is so commonly observed in the horse tissue, does not occur in the mule. The nearest approach to it is a small clump of threads which is frequently seen and seems to be formed in the position occupied by the large karyosome. This clump persists in many cells until the threads break up into chromosomes. The mass of chromosomes resulting from such a clump of threads can also be frequently seen. It is probable that the clump of threads is brought about by the tangling up of the linin strands, which extend from the karyosome.

The spireme stage which follows synizesis and synapsis in the horse is also lacking in the mule. The expanded network takes its place, and only parts of the network resemble the spireme of the

horse. Even in cases where long threads occur the resemblance is slight, for invariably the thread is made up of portions of various thicknesses, while the spireme in the horse is of a more uniform diameter.

### 2. *Synapsis.*

It appears that thus far there was no necessity for the paternal and maternal chromosomes in the cells of the hybrid to coöperate to any noticeable extent in functioning, and while some conflicting tendencies may be in operation, each group mixed with the other, seems to have gone on performing its functions normally. Material from both parents undoubtedly prevails in the somatic cells of the mule as it does in the sex cells. Yet these materials in the somatic cells, coming from two vastly different animals, do not in the least interfere with each other physiologically. And while no somatic tissue of the mule has been studied, any suspicion of abnormalities, such as disintegration and decay for example in the muscle cells, could hardly be substantiated by our knowledge of the health, strength, endurance, and longevity of these hybrids. This suggests that developmental processes are also normal. It is highly probable, then, that since the physiological and developmental processes are normal that the mechanism of cell division during the process of development and growth is also normal; the same as it is in the spermatogonial cells where perfect mitotic figures appear and all of the chromosomes divide.

The real conflict ensues during the various stages of the primary spermatocyte as is so plainly evidenced by the numerous abnormalities which occur in these cells. In fact the conflicting tendencies are so great that the destruction of each cell is inevitable, and no spermatozoa are produced, causing the hybrid to be sterile. Up to this stage the paternal and maternal plasmas evidently retain their individuality and would undoubtedly continue to do so were it not for the wonderful phenomena of reduction, which necessitates a fusion of the chromatin components of the germ cells at this stage of maturation, as is known in many of the normal forms in which spermatogenesis was carefully studied.

It is now a well-known fact that in maturation of the germ cells a reduction of the ordinary number of chromosomes to

one-half takes place. Before this actual reduction, there is usually a so-called pseudo-reduction, in which the chromosomes unite in pairs so that when the cell is ready for division, although only half of the regular number of chromosomes appear, each is really double or bivalent and equivalent to two of the single or univalent type.

In this hybrid, as was suggested before, it may be supposed that in the somatic cells and in the spermatogonia, the chromosomes from the paternal and maternal animals lie side by side and carry on the customary functions of the cells; but when it comes to an actual fusion of the chromosomes to form the bivalent type necessary for reduction, the incompatibility of the two plasmas renders the pairing difficult and incomplete, or prevents it entirely.

In the horse pairing or pseudo-reduction takes place during the period of synizesis. This period is characterized by massing of the chromatin threads in the center of the nucleus, and later the nuclear wall expands and the entire mass passes to one side of the nucleus leaving a large clear area in the remaining portion (Wodsedalek, '14). This condition is much the same as in the pig (Wodsedalek, '13), except that in that animal the nucleoli were invariably found within the mass of threads and in a position nearest to the nuclear wall; while in the horse the nucleoli are almost invariable within, or next to the clear area. Shortly after the collapse of the chromatin material, the threads pair and appear in apparently half the original number and twice as thick. Later they expand and again occupy the entire contents of the nucleus. Then follows the period of growth, and eventually the threads break up into the bivalent chromosomes which appear in half the original number found in the spermatogonia, plus the accessory.

The pairing of the various components, in the horse, takes place simultaneously, and when the spireme appears it possesses a fairly uniform diameter throughout. This fact indicates clearly that each portion of the spireme is of a bivalent nature, for should some parts remain unpaired or univalent in nature, such parts could be readily discerned by the sudden and noticeable decrease in their diameters. No pairing of parts in the horse

was ever observed after the synizesis period, and no chromosomes ever seem to remain unpaired in the primary spermatocyte, with the exception of the accessory which is always unpaired and passes undivided to one pole or the other.

In the mule the period of synapsis is extremely fascinating, and the facts presented here will no doubt stimulate many investigators in the study of hybrid sex cells. The author considers himself exceedingly fortunate in being able to obtain hybrid material which was in such excellent condition for this investigation, especially since the hybrid in question is the offspring of two vastly different parents. Thus far, it appears that there is no case on record among the vertebrates, showing that this important step in the process of maturation of the sex cells of hybrids has been fully and conclusively demonstrated; and since this important phenomenon has never been clearly demonstrated in a hybrid of this nature, and since this material given to me by Dr. Kalkus appeared promising from the start, the author spared no time and energy in making a careful and prolonged study of this particular phase in the maturation of the sex cells of the mule.

It might be stated at the outset that little fusion of the chromatin material takes place, and that there is no definite time for such fusion. As soon as the various chromatin threads in the network of the nucleus become well defined, there appear indications of pairing of some of the threads. This process continues even after the network breaks up and the chromosomes make their appearance (Figs. 8, 9, and 12). Fig. 8 represents an early stage in which two or three of the threads had fused, and a beautiful case of two long threads lying side by side almost across the entire diameter of the large nucleus. Fig. 9 shows a cell in which many more threads had fused and some are still in the process of fusing. Fig. 10 shows a case where only a small amount of fusion had taken place before the parts of the chromatin network had become loose; but the process of pairing seems to have continued in two or three instances regardless of the fact that some of the chromosomes were in the stage of disintegration. Fig. 11 represents a more advanced case, where apparently a great deal of the chromatin material had fused

before the network broke up and still there are indications of pairing. Cells of this nature in which a great deal of fusion had apparently taken place, invariably show more or less pronounced indications of decay, and the question arises as to whether this unusual amount of fusion is due to the condition of decay or whether the degeneration sets in because of the unusual amount of fusion. It appears, however, that the great amount of fusion is caused by the existing degenerate condition of the cell in general, for invariably masses of chromatin material bearing no resemblance to normal chromosomes or threads are present in these cells. Fig. 12 shows a still further advanced stage, in which conjugation continues.

There is no regularity in the amount of fusion that takes place in the various cells. In some cells many more chromosomes conjugate than in others. This fact was apparent during the various stages of the comparatively long synaptic period, and became more obvious when numerous accurate counts of chromosomes were made in cells in which all indications of pairing had ceased. The fact that the nucleus expands enormously during the "spireme stage" was a great aid in making many accurate counts. The chromosomes, as a rule, were well segregated with only partial overlapping. Here and there were tubules with cells in exceptionally good condition for this particular investigation, thus rendering possible many definite counts. The number of chromosomes in the different cells at this stage vary considerably. The smallest number found was thirty-four and the largest was forty-nine. This seems to indicate without a doubt that in the few cases where only thirty-four chromosomes could be counted that thirty-two of the fifty-one univalent autosomes had fused. In the few cases where as many as forty-nine counts were made it appears equally as certain that only four of the fifty-one chromosomes had fused. It must be remembered, however, that these two extreme counts were obtained in only a very few cases and stand out as exceptions. All other counts between thirty-four and forty-nine were obtained, but by far the greater majority of counts lie between forty and forty-five, with a fairly good number of thirty-eight and forty-six.

A very interesting as well as important feature about the

chromosomes at this stage is the fact that the bivalent ones can as a rule be readily distinguished from the univalents, and the loss of univalents can be accounted for by a proportional increase of the bivalents. If, for example, forty-one univalent chromosomes can be detected, it means a loss of ten others. This loss of ten univalent chromosomes can usually be accounted for by the presence of five large or bivalent chromosomes, making a total of forty-six. If only thirty-one chromosomes can be counted, which means the disappearance of twenty univalents as such, one can usually find ten large bivalent chromosomes, making a total of forty-one. This part of the problem was so fascinating that the chromosomes of hundreds of cells, in which they were well scattered, were carefully counted and an attempt was made to account for the loss or gain, depending on the type of chromosomes that were considered first. In some cases those which appeared to be univalent were counted first and then the bivalents. In other cases those which appeared to be bivalent were counted first and then the univalents, and it is surprising in what a large percentage of cases the necessary number of fifty-one, in terms of univalents, was actually obtained.

Occasionally a discrepancy of one or two, and in rare cases, three bivalent chromosomes, was noted, or in other words, it was sometimes impossible at first sight to distinguish all of the bivalent chromosomes. Even in cases when the individual chromosomes were carefully examined in regard to their single or double nature, one could not always be positive whether he was dealing with a single or a compound body. This fact, however, should not be so very surprising because two of the smallest type of single chromosomes may fuse to form a bivalent one of practically the same size as some of the larger univalent chromosomes. The matter suggests rather strongly that it is not always the same chromosomes that fuse, even in cases where the same counts are obtained. On the other hand there is a possibility of some of the large chromosomes being tri- instead of bivalent, since the fusion of three threads, as well as three chromosomes, was observed in several cases (Fig. 12). In cells in which decay is noticeably under way it frequently occurs that many chromosomes fuse together and form a large body, but this cannot be regarded as fusion in the same sense as synapsis.

It can now be clearly seen that great variation exists in the amount of fusion which takes place in the chromatin material of the various cells in this stage of the maturation process. In some cells fully eight times as many chromosomes pair as in others. The question at once arises why should such a decided variability exist in this respect. It is not very probable that the various cells resulting from the last spermatogonial divisions differ so greatly in their make-up, as no such variability was observed in the spermatogonial cells themselves. The fate of the different primary spermatocytes with respect to the extent of pseudo-reduction of their chromatin material is evidently determined at the time of the formation of the network of the linin threads, for these linin threads seem to persist as the axes of the later chromatin threads which eventually break up into chromosomes.

The nature of the cause of the linin strands connecting with each other in such confusion as they are found, must necessarily remain a matter of conjecture at present. The same force which in normal sex cells at this stage causes the linin strands to come in contact at their free ends, thereby forming long threads, or loops, or a continuous strand, is apparently brought into action in the hybrid cells but not with the same precision. The fact that the two plasmas are so different apparently causes the strands to connect with each other at any point and at any angle, instead of at their free ends only, and thereby forming a continuous network rather than long strands. The tendency of the strands to connect with others, however, must be well pronounced for very seldom can any free ends be seen in the earlier stages (Figs. 8 and 9). Later on, of course, free ends make their appearance but these are caused by the breaking up of the network in parts rather than original failure at fusion. In some cells the end to end fusion of the original components is more pronounced, since many long threads appear in such cases. However, not a single case was seen in which several of the cross-connecting pieces were not present, which condition was never observed to occur in the corresponding stages in the sex cells of the horse.

The fact that the ends of the threads are fused on to some other part of the chromatin network seems to be a drawback

in their fusion. Frequently two threads, which have some affinity for each other, and lie in a position parallel to each other and are not too taut, fuse along the central portion with the ends remaining in the shape of V's, because the two threads are attached at each end some distance apart. At times when parts having an attraction for each other are so arranged as to form an acute angle, fusion begins at the point of the angle and continues a short distance, leaving a V at one end. The ends remain unfused at that point, until the network breaks up into chromosomes, when the temporary connections of the two chromosomes in question, too, are severed, and thereby complete their fusion.

There is also considerable evidence that actual migration for purpose of fusion of some parts of the net-work takes place before the connections are entirely severed, and even before there are any noticeable indications of such connections separating, for frequently we find a bivalent thread with both ends still attached to other threads.

### 3. *Abnormalities in Mitosis.*

The abnormalities in mitosis bear a striking resemblance to the abnormalities found in the sex cells of hybrid pigeons by Guyer ('00). In speaking of abnormalities in pigeons, Dr. Guyer says: "The abnormalities in mitosis are in the nature of multipolar spindles and asymmetrical division and distribution of the chromosomes (Figs. 28–39). These may exist independently one of another, or both may occur together in the same cell. They are more pronounced in sterile birds but may at times be seen in the fertile forms. In very many of the division of primary spermatocytes one or the other, or both of these phenomena are seen. It is a curious fact that the multipolar spindles seem to be confined largely to the primary spermatocytes, and one is prompted immediately to associate the fact with the pseudo-reduction or formation of bivalent chromosomes which occurs normally at this stage of spermatogenesis. The irregularities in chromatin distribution are also seen for the most part in the primary spermatocytes."

The abnormalities in the mule are also in the nature of multipolar spindles and there are attempts at asymmetrical division and distribution of the chromosomes; but in addition to these

types of abnormalities we have cases of scattered chromosomes which do not enter into the spindle at all (Figs. 16–36). Guyer's suggestion regarding the curious fact that the multipolar spindles seem to be confined largely to the primary spermatocytes prompts one immediately to associate the fact with the pseudo-reduction or formation of bivalent chromosomes which occurs normally at this stage of spermatogenesis, is considerably strengthened by the results of this investigation on the mule, since a thorough study of the entire phenomena of pseudo-reduction was possible in this hybrid.

The diverse types of multipolar spindles and other abnormalities that occur in the primary spermatocytes of the mule are shown in Figs. 16–36. Fig. 16 represents a polar view of a metaphase stage. Twenty-eight chromosomes, some bivalent and some univalent, can be seen in the equatorial plate, while sixteen all apparently univalent, together with the accessory are scattered about in the cytoplasm. Fig. 17 shows a meager attempt at spindle formation. A bunch of chromosomes is found at the equatorial plate and only a few spindle threads could be seen and those appeared to be broken up. There was no sign of the centrosomes being present. The other chromosomes, both bivalent and univalent, are scattered about. The cell was no doubt in the process of decay. A trivalent chromosome can be seen at the lower part of the figure. Trivalent chromosomes are not uncommon but the three components can seldom be seen at this advanced stage. They usually fuse together, forming a large chromosome, frequently spherical in shape. Especially is this true when the cell is in the process of deterioration. The large spherical body at the upper part of Fig. 18 was probably formed in this way, though it is possible that it is composed of more chromosomes.

Fig. 18 represents a cell similar to that shown in Fig. 17. These cells were found together with many other similar cells in the same tubule, and several tubules bearing such type of cells were observed. All of these cells were characterized by a large clump of chromosomes, which was presumably an attempt at plate formation, and a large number of chromosomes, uni-, bi-, tri-, and even quadrivalent, are scattered about the entire

contents of the cell. The cytoplasm of these cells was invariably degenerated; the remains being closely assembled about the large clump of chromosomes, leaving the periphery of the cell clear in appearance. The cell wall, however, retained its normal outline up to this stage of degeneration, which seems to suggest that the clear space was occupied by a fluid. While there were indications of spindle threads more or less pronounced in some cells, many of the cells showed no signs of the presence of such threads (Fig. 18). In such cells they either disappeared or had never been formed. It is a curious fact that such type of cells usually appeared in large numbers in the same locality and that only several tubules bearing them were found, though occasionally single individuals of that type were found in other tubules. The heart-shaped accessory could usually be seen somewhere off by itself (Fig. 18).

Figs. 19–21 show a common type of freak in which the spindle is bipolar and a number of chromosomes did not find place in the equatorial plate but remain scattered about, either somewhere on the spindle or in the cytoplasm. Figs. 19 and 20 each show the large accessory at one pole. In Fig. 21 the accessory could not be easily detected.

Fig. 22 shows an uncommon type of unequal division in which five large chromosomes have passed over to one pole, while about thirty-six remained in the original place. This indicates that the total number of chromosomes in that cell is forty-one and that ten of them, in view of the previous evidence and discussion, must necessarily be bivalent. Each of the five at one pole on account of their size appear to be bivalent. One of them, in which the two parts have not fused along their entire lengths, obviously shows its bivalent nature. Further evidence that these five are double can be based on the fact that only about five of the chromosomes in the big group appear to be double. This shows then that no chromosomes have divided, but that five bivalent ones were pulled over to one pole in their entirety. Each of these groups without further division would in all probability, if decay did not set in too early, form a separate nucleus. And it can be safely assumed that the binucleated condition of cells sometimes seen in this tissue, in which one nucleus is much larger than the other, was brought about in this fashion.

Fig. 23 shows still another type of cell with a bipolar spindle or an attempt at one. A ring of ten chromosomes can be seen near one pole and partly pushed out of the cell through the cell wall which had been broken at that place. Judging by the convergence of the spindle threads in that half of the cell one would conclude that these ten chromosomes never occupied a position in the plate, and it appears as though this was an attempt on the part of the cell to get rid of some of the chromatin material which did not harmonize with the other parts of it. The clump of chromosomes was apparently repelled by the others with sufficient force to cause them to break through the cell wall. Only about twenty other cases similar to the one just described were observed in all of the tissue studied. However, a number of these the author is inclined to regard, on account of circumstantial evidence, as being due to methods of technique, though even in the more suspicious cases he is not absolutely positive that such in the real cause. In at least a dozen of cases there seems to be no doubt but that the expulsion of masses of chromosomes is actually due to the activity of the cell itself. Among these twelve cases a regular series from partial to total expulsion of a mass of chromosomes can be demonstrated. The number of chromosomes comprising such a discarded mass varies from five to nineteen.

The assumption that an effort is made on the part of some cells to discard some of the chromatin material is further substantiated by the fact that in many cells a mass of chromosomes was seen closely applied to the inner side of the cell wall. At times the cell wall bulges out at such a point and has the appearance of being quite taut in the immediate vicinity of the chromosomes, giving the impression that some repelling force is acting upon them. The most notable evidence in favor of this assumption lies in the morphology of the cell, represented in Fig. 24. The unfortunate thing about this matter is, of course, that only a single cell of its kind, the one here represented as accurately as possible, was found even after a long and diligent search for more was made. It may at first seem very unscientific or even ridiculous to attach so much value to a single cell. Nevertheless, the significance of the structure of this cell, lonely as it is, must

not be underestimated. The cell is undoubtedly a primary spermatocyte in the telophase stage. The cytoplasm has failed to divide. This is in accordance with all other observations made on primary spermatocytes in this hybrid, since not a single case was noted in which the cytoplasm showed even the slightest indications of division or construction.

Just exactly what the nature of the cell was in its metaphase stage with regard to the arrangement of the chromosomes can not be definitely stated. One thing seems fairly certain, and that is that no division of individual chromosomes took place. For the number of chromosomes can still be fairly accurately determined since they had become only partially disintegrated. A count of the chromosomes in the various parts of the cell reveals a total of about forty-three, fourteen in the oblong nucleus, ten in the spherical one, three in the cytoplasm, and sixteen in the small spherical nucleus outside of the cell proper. The total of forty-three shows that eight chromosomes are lacking to make the total of fifty-one contributed by the last spermatogonial division. In accordance with the prevailing scheme of synapsis in other primary spermatocytes this means that eight of the forty-three chromosomes should be bivalent and thirty-five univalent. In examining the various chromosomes with regard to size, nine of them seem to be noticeably larger than the rest; five are in the oblong nucleus and four in the large spherical one. One of the nine is possibly the accessory, thus leaving the required number of eight bivalent chromosomes. The three left behind in the cytoplasm and the sixteen outcasts are all univalents.

As to the origin of the two nuclei within the cell, several possibilities suggest themselves. The possibility which appears most tenable is that the cell in its metaphase stage resembled the one represented in Fig. 32. It can be seen from that figure that the cell possessed a large spindle, at one side of which was a plate of chromosomes with threads extending only to one pole. In another part of the cell is a group of about twenty chromosomes with no signs of spindle formation. It can be assumed that the two nuclei shown in Fig. 24 were formed from two masses of chromosomes resembling the two plates of chromosomes shown in Fig. 32, and that the nuclei were reorganized

without division of the chromosomes. The latter phase of the assumption is based on the fact that indications of the actual division of the chromosomes in the primary spermatocytes are extremely rare, and that occasionally it can be seen that the nuclei actually enter the process of reorganization before the chromosomes had divided. This is especially true in cells with multipolar and double spindles. As to the nucleus outside of the cell (Fig. 24), it may be suggested that the chromosomes contained therein resembled in the metaphase stage of this cell the group of chromosomes without spindle threads represented in Fig. 32, and that later they were expelled from the cell after the fashion shown in Fig. 23. The three chromosomes in the cytoplasm were probably treated with indifference by the three large groups of chromosomes, being neither attracted nor repelled by them, and thus remained scattered about in the cytoplasm. The material necessary to construct a separate nuclear wall for the three deserted chromosomes was apparently lacking.

Another possibility as to the origin of the nuclei in question is that the cell may have had two spindles, as are shown in Fig. 29, with an additional group of chromosomes without the spindle threads, for such cells were also observed. Still another possibility is that the cell may have possessed three spindles like those shown in Fig. 34 and that the chromosomes belonging to one of the spindles were eventually expelled from the cell and there reorganized a nucleus while the other two spindles remained within the cell.

There seems to be no doubt but that the chromatin material found in the small nucleus on the outside of the cell shown in Fig. 24, originally formed a part of the inner contents of the same, for a distinct connection in the form of coarse granular threads still persists between it and the chromosomes within the cytoplasm, as well as the two large nuclei themselves. The group of sixteen chromosomes was no doubt expelled from the cell and there formed a separate nucleus. The nuclear membrane is thin but well defined. It can also be seen that a quantity of cytoplasm was forced out with the chromosomes. Some of it, very finely granular, persists around the nuclear membrane. The rupture in the cell wall occasioned by the expulsion of such

a mass of chromosomes had evidently regenerated in this cell. That the cell had begun to degenerate is evidenced by the presence of large vacuoles and other irregularities in the cytoplasm. The signs of decay always seem to be manifest in the cytoplasm first and then in the chromatin.

The fact that more cells of exactly this type could not be identified is no doubt due to decay which almost invariably sets in before the aim of the cell is accomplished, as will be pointed out later. It is evident that at least some of the other cells seemed to act in the same direction but succumbed before the reorganization of the nuclei could take place. Then, too, it seems reasonable to believe that the isolation of a particular group of chromosomes, as a necessary preliminary step to the function of expulsion, depends largely on the relative arrangement of the linin strands representing such a group of chromosomes in the early stages of these cells. According to our knowledge of the extreme variability in the structure of the linin strand network, such a favorable arrangement of the unwelcome chromatin material can possibly be regarded as being accidental.

Judging by the appearance of the chromosomes, the author is inclined to think that the material expelled by the cells is largely that which was contributed by the mother of the hybrid; and while the number of chromosomes so expelled varies in the different cases, nineteen chromosomes including the accessory which is the number presumably of maternal origin were found in a few instances. There were never more than nineteen chromosomes in the expelled group. This, however, is only a matter of suggestion, for the situation can not possibly be cleared up until a careful study has been made of the sex cells of the ass.

Fig. 25 shows a type of cell in which there is one large spindle and a bunch of chromosomes forming a small plate located at right angles to the large one. It can be seen that a number of threads radiate from the chromosomes in the small plate and that some of them extend to the poles of the large spindle. The large chromosome in the upper part of the spindle is the accessory. Fig. 26 shows a peculiar quadripolar spindle. Most of the chromosomes are in the plate of the large spindle and many of them show signs of division. A small group of chromosomes is located

at right angles to the right of the large plate, and spindle threads, extending from them and converging at a conspicuous centrosome, could be plainly seen. On the other side some of these chromosomes seem to be connected by threads to the one pole of the large spindle and some to the other. At the upper pole of the large spindle is a group of eleven chromosomes with spindle threads converging at a centrosome near the cell wall. There was a slight indication of threads on the other side passing to the upper centrosome of the large spindle, but these could not be definitely made out since the spindle in question was at a somewhat oblique angle to the large spindle, and the threads on the opposite side were obstructed from view by the group of eleven chromosomes. The centrosomes in this cell are unusually large and conspicuous. The large chrosome at the lower portion of the main spindle is the accessory.

Fig. 27 is apparently another attempt at a double spindle, the small one at right angles to the large. There was no sign of centrosomes. Fig. 28 is a polar view of a cell with two spindles. In each case the small chromosomes are in the center of the plate. Seventeen chromosomes form the plate in one spindle and twenty-five in the other, making a total of forty-two. There then should appear ten large chromosomes, including the accessory in this cell. In the smaller spindle containing seventeen chromosomes, four are undoubtedly bivalent, and in the large spindle of twenty-five chromosomes, six should be larger than the rest, which upon very careful examination, seems to be the case, but some of the other chromosomes are also somewhat larger than usual. This condition is possibly due to the fact that decay is well under way in the cell and the chromosome may have become distorted.

Fig. 29 shows an excellent case of two spindles, one is larger than the other and also contains the larger chromosomes. It is probable that the large spindle is paternal in nature, and the small one maternal.

Fig. 30 shows an unusual freak with the upper part giving the appearance of two closely applied spindles. At the opposite end, however, the threads do not converge at two separate poles nor at a single pole but remain loose. At first sight one gains the

impression that the spindle was a tripolar one, with two poles on one side of the double plate and a single pole on the opposite side, and that the single pole was cut off in the process of sectioning. But a careful investigation shows that the cell is entire and that no part of it was cut off. Guyer ('00) represents a similar case in hybrid pigeons, and describes it as a case in which each fiber of the unusually loose spindle seems to terminate at one end in a small centrosome-like dot or granule.

Fig. 31 shows a tripolar spindle which is really a case of two spindles fused together. All of the chromosomes are found on the spindle and some are apparently in the process of division. Fig. 32 shows another type of tripolar spindle and a group of nineteen chromosomes, including the accessory, in the cytoplasm, corresponding to the group of chromosomes which are sometimes expelled from the cell. Fig. 33 shows a degenerate cell with three ragged spindles converging at a common point. The accessory chromosome is off by itself and from it extends a thread toward the point of convergence of the three spindles. The cell is well along in the stage of decadence. Some of the spindle threads are broken up and others seem to have fused together.

Fig. 34 shows a cell with three perfect spindles meeting at a common conspicuous centrosome. The bivalent chromosomes appear to be distributed among the three spindles. Fig. 35 shows an extremely rare type of quadripolar cell with five spindles closely and symmetrically arranged, with three chromosomes remaining loose in the cytoplasm. Fig. 36 shows a cell which is the only one of its kind that was found and is no doubt the anaphase stage of a cell with a quintespindle arrangement similar to that represented in Fig. 35. Eighty-five chromosomes, including the accessory near the centrosome to the left, could be distinguished as represented. Six of the chromosomes in the central spindle evidently had not divided. This in terms of univalence would suggest that there is a total of ninety-one chromosomes in the cell. The accessory remains undivided as it does in the horse in the primary spermatocyte. Without the accessory, then, there would be ninety chromosomes, which suggest rather strongly that forty-five autosomes, of which five were presumably

bivalent lined up for division in the metaphase stage of this cell and that all but the six in the center had divided. Only two or three of these six remaining undivided could possibly be taken for bivalents, which fact indicates that the bivalents as well as the univalents had divided. The centrosomes were well defined and there were only slight indications of deterioration in the cell.

The researches of Hansemann ('91), Lustig and Galeotti ('93) and others, show that asymmetrical mitoses are of very general occurrence in carcinoma cells and other pathological tissues; and Schottlander ('88) and Galeotti ('93) among others have shown that similar abnormalities in mitosis may be produced artificially in many tissues by treatment with dilute solutions of various drugs, such as quinine, chloral hydrate, cocaine, potassic iodide and antipyrin.

At first thought, in view of what is known about the effects of drugs on mitosis, it would appear that the abnormalities in division of the primary spermatocytes are probably due to some deleterious chemical substances which may be produced by the degenerative processes going on in the tubules. This however, does not account for the fact that the abnormalities in the mule are practically entirely restricted to the primary spermatocytes. It appears more certain that the abnormalities in this hybrid are due to the conflicting tendencies brought into action within the cells themselves at this stage of spermatogenesis when coöperation between the two parent plasmas becomes necessary but is impossible on account of their vast dissimilarity.

## Giant Cells.

Giant cells appear occasionally but do not seem to be as numerous as was described by Guyer ('00) in hybrid pigeons. Fig. 37 represents a large cell of rare occurrence. It appears to be a primary spermatocyte with three spindles in the anaphase stage, but the chromosomes are unusually small and resemble more those of the nurse cells.

Fig. 38 shows an enormous cell with four large nuclei. The cell is evidently a primary spermatocyte in the early prophase, as the size and structure of the nuclei, and the uniform consistency of the cytoplasm are identical to those of normal cells in

this stage (Fig. 7). The abnormality appears to have taken place in the last two divisions of a spermatogonial cell; only the nuclei have divided and therefore remained in the same mass of cytoplasm. Primary spermatocytes with four nuclei appear occasionally, but in practically all cases there is evidence that the abnormality had occurred within the cells themselves and not in the spermatogonia.

Fig. 39 is another giant cell in which all of the chromosomes seem to be in the huge non-symmetrical quadripolar spindle. About a hundred chromosomes could be counted in this cell, some of which are apparently the result of division and others are in the process of dividing. There are undoubtedly many more chromosomes present but the exact number could not be determined owing to the position of the large equatorial plate. It is probably a spermatogonial cell which possessed two complete nuclei and the number of chromosomes in excess of one hundred and two may be due to the fact that many of the chromosomes have divided. Parts of the spindle seem to be in the late metaphase and others in the anaphase stage.

### Multinucleate Cells.

Primary spermatocytes with nuclei ranging from two to six in number are not uncommon (Figs. 40–43). The most common ones are those with two and four nuclei. Such cells appear to be in the late telophase stage. There is much irregularity in size among the nuclei of the same cell. For example, when two nuclei are present they are sometimes of similar size and then again one is much larger than the other. In cases where three nuclei are present they are sometimes of practically the same size, or form a gradation in sizes, or two large and a small one, or two small and a large one appear, and so on. Frequently one or more chromosomes were left in the cytoplasm while the others formed one or more nuclei. Fig. 41 shows a decaying cell with two nuclei, and a large chromosome left in the cytoplasm but connected with one of the nuclei by a coarse strand. Such strands were not always present. It is possible that the chromosome in question is the accessory, though one of the chromosomes within the nucleus to the left also resembles it.

The formation of more than one nucleus is not at all strange, when we consider the various freakish spindles of the primary spermatocytes and the fact that the cytoplasm of these cells was never observed to be in the process of division. A spindle like those shown in Figs. 34–36 for example, may, if decay does not set in beforehand, form five or six nuclei. A cell like that represented in Fig. 29 may eventually possess four nuclei, one like that shown in Fig. 31 three nuclei, and so on. In all of the comparatively few cases that reach the telophase stage before decaying, we must necessarily expect some such abnormalities.

## Destruction of the Germ Cells.

In speaking of degeneration of the germinal cells in hybrid pigeons, Guyer ('10) says: "Degenerative processes were in progress in the testes of all the sterile forms, but were most pronounced in hybrids between very divergent species, or between unlike hybrids from birds which were themselves descendants of fertile hybrids. There were some such extreme cases of degeneration that only the layer of cells lying along the wall remained in the tubule. Where such a degree of degeneration exists there is, of course, no approach to the formation of spermatozoa. There is often a strong invasion of wandering cells into the tubules, especially where the degenerative activity has become extensive. The interspaces between such tubules are also usually packed with cells which have much the same appearance as white blood corpuscles. Little clumps and globules of deeply staining cytoplasm are scattered about among the cells within the tubules. The oval nuclei of the Sertoli cells are also generally to be seen in varying numbers.

"In some places it really looks as if the germinal cells themselves lose their cell walls and characteristic appearance and become leucocytes, but this point will require very careful study before any definite conclusions can be reached. In other tubules the original cells, or cells which have wandered in, settle down and take on exactly the appearance of the large stroma cells which are ordinarily present outside the tubules. The cytoplasm of such cells has a peculiar alveolar-like appearance that is very characteristic."

The destruction of the sex cells in the mule appears to be restricted to the primary spermatocytes, where it seems inevitable. The incompatibility of the two widely different plasmas contributed by the parents renders completion of spermatogenesis in this hybrid impossible and the animal remains sterile. The process of deterioration sets is as soon as coöperation of the chromatin material becomes necessary in the primary spermatocyte. By far the largest amount of destruction takes place in the "spireme stage" before the chromosomes can make their appearance. Sometimes the nucleus in this stage becomes enormously distended and the thin chromatin threads break up into numerous fragments before any noticeable pairing takes place. Then again, the fragmentation takes place after some of the threads fuse (Fig. 10), or after the chromosomes make their appearance (Fig. 44).

The cytoplasm seems to decay first and large masses of naked nuclei in various degrees of decadence can be seen in some of the tubules. Small masses of chromatin material, pieces of threads, cytoplasm, and cell wall, are also abundant in many of the tubules. Occasionally small, naked, though well-defined nuclei are seen. Whether these are formed from cast-off material like the one shown in Fig. 24, or whether they are small nuclei similar to those shown in Fig. 42, retaining their structure after the cytoplasm had fallen to pieces is difficult to determine. The latter suggestion can easily be conceived, and the former is not unreasonable, since there is no logical reason to make one believe that the expelled material invariably remains attached to the cell. A much more legitimate assumption would be that, since the material is cast off by the cell, it ordinarily leaves the cell entirely and becomes free among the other material in the lumen of the tubule. This assumption would tend to explain further the extremely rare occurrence of cells similar to the one shown in Fig. 24.

Figs. 44–52 show various types of degenerating cells. Fig. 44 is in the late prophase, and Fig. 45 in the metaphase showing a partly expelled mass of disintegrating chromosomes. Figs. 46, 48 and 49 show the collapsed condition of the cytoplasm and the dense nature of the nuclei. The nuclei sometimes retain their

normal shape but the chromatin material within fuses into a continuous mass, which is often perforated by many vacuoles (Figs. 46 and 49). Occasionally a single large vacuole appears in the center of the nucleus leaving the chromatin material arranged in a layer next to the nuclear membrane (Figs. 48 and 52).

Fig. 47 is a degenerate cell possibly in the late prophase stage in which many of the chromosomes have fused into large spherical bodies which remained scattered about in the cytoplasm. Fig. 50 shows a cell with an ill-formed nucleus and a mass of chromatin material left out in the highly degenerated cytoplasm. Fig. 51 shows a cell in an advanced stage of destruction in all of its parts. Fig. 52 shows a collapsed nucleus with a portion of naked cytoplasm.

On account of the broadcast destruction of the primary spermatocytes in the early stages, a comparatively few are left that attain the metaphase and early anaphase stages (Figs. 18–45) when they, too, meet their fate; and the remaining few that survive the anaphase succumb soon after, and no secondary spermatocytes nor spermatids, and consequently no spermatozoa are formed and the hybrid therefore remains sterile.

The invasion of tubules by wandering cells is not as pronounced in the mule as in pigeon hybrids, as described by Guyer ('00). Even where the degenerative activity was most pronounced the leucocytes were rare or absent altogether. Nor was there any indication of the germinal cells themselves losing their cell walls and characteristic appearance and becoming leucocytes, as sometimes appears to be the case in hybrid pigeons according to the same author.

## Summary.

1. The parents of the mule are widely different as can be seen from the comparison of the horse and the ass.

2. The mule partakes of both the sire and the dam, but appears to resemble the ass more than the horse, both in structure and habits.

3. The greatest difference seems to lie in the relative number of chromosomes in the cells of these two animals. The horse has thirty-seven and the mule fifty-one. This suggests that the

number in the ass is about sixty-five, thus making a difference of twenty-eight chromosomes between the parents of the hybrid.

4. The seminiferous tubules of the mule contain a much smaller amount of germ cells than do the tubules of the horse. Some of the tubules of the mule are entirely devoid of sex cells.

5. The sex cells of the mule are larger than those of the horse in the corresponding stages.

6. All of the fifty-one chromosomes in the spermatogonial cells of the mule enter the spindle for division. The mitotic figures are normal and there are no straggling chromosomes.

7. The accessory chromosome of the hybrid, which is undoubtedly maternal in origin, resembles entirely the accessory of the horse, which fact shows that this sex-determining chromosome retains its individuality.

8. The period of synizesis which is so obvious in the primary spermatocytes of the horse, is lacking in the mule.

9. The spireme of the horse is also lacking in the mule, but is replaced by a continuous network of chomatin threads, parts of which sometimes resemble the spireme to a certain extent.

10. There is no definite time for the pairing of threads or chromosomes in the hybrid; the synaptic period begins at the time the chromatin threads make their appearance and continues through the prophase.

11. The pairing of chromosomes, or pseudo-reduction, is always incomplete and very inconstant. The number of chromosomes in the late prophase of the primary spermatocytes varies from thirty-four to forty-nine. The greatest majority of counts of chromosomes lie between forty and forty-five. The expected number, if reduction was complete, would be twenty-five besides the unpaired accessory.

12. In the late prophase of the primary spermatocytes the bivalent chromosomes can as a rule be readily distinguished from the univalent ones. The number of chromosomes which the various cells lack in order to make the original total of fifty-one, in terms of univalence, can usually be accounted for by the proportional increase in the presence of bivalents in such cells.

13. Up to the early prophase of the primary spermatocytes there seems to be no necessity for the paternal and maternal

chromosomes to coöperate in functioning. Each group seems to go on performing its functions normally. The real conflict ensues during the various stages of the primary spermatocyte, and is no doubt occasioned by the necessity for coöperation on the part of the paternal and maternal chromosomes in the process of conjugation or pseudo-reduction.

14. Abnormalities in mitosis occur invariably in primary spermatocytes that attain the metaphase stage.

15. Giant cells are occasionally seen.

16. The chromatoid body which is very conspicuous and constant in the horse is entirely lacking in the mule.

17. There is considerable evidence that primary spermatocytes make an attempt to eliminate some of the chromatin material.

18. The chromosomes expelled by the cells appear to be those which were contributed by the mother of the hybrid.

19. Destruction as well as abnormalities in mitosis seem to be restricted to the primary spermatocytes. Most of the cells disintegrate during the prophase, especially during the period of synapsis. Others meet their fate in the metaphase or early anaphase stages. The remaining few that survive the anaphase succumb soon after, and no secondary spermatocytes nor spermatids, and consequently no spermatozoa are formed and the hybrid remains sterile.

20. There are no authentic cases on record showing that fertility ever occurs in this hybrid.

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## EXPLANATION OF PLATES.

### PLATE I.

(All of the drawings were made with the aid of a camera lucida, × 3,000.)

FIG. 1. Early spermatogonial cell showing a large heart-shaped nucleolus and two small karyosomes.

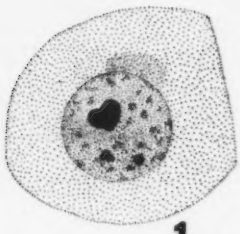
FIG. 2. Resting stage of a full grown spermatogonial cell showing the large triangular or heart-shaped nucleolus and the two spherical karyosomes.

FIG. 3. Late prophase of spermatogonial division showing fifty ordinary chromosomes and the large accessory which can easily be distinguished.

FIG. 4. Metaphase of division in a spermatogonial cell showing a perfect spindle with the accessory dividing at the left.

FIG. 5. Anaphase of division of a spermatogonial cell showing a perfect mitotic figure.

FIG. 6. Late anaphase of division of a spermatogonial cell with perfect mitosis again in evidence.



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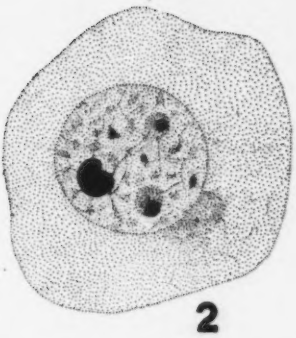

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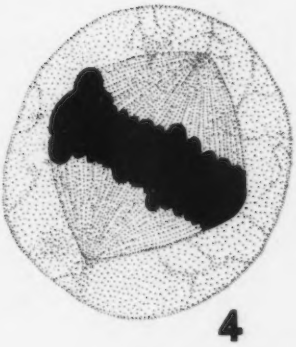

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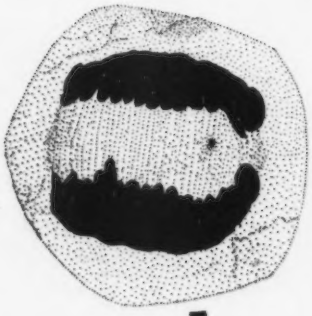

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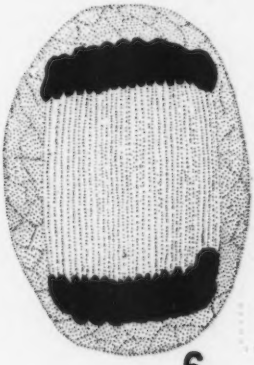

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PLATE II.

FIG. 7. Resting stage of a primary spermatocyte showing the characteristic heart-shaped nucleolus and the karyosomes from which radiate fine linin strands.

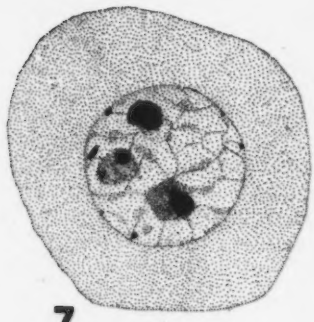
FIG. 8. Primary spermatocyte showing a continuous network of chromatin threads, some of which have paired and others are in the process of fusion, but most of them are unpaired.

FIG. 9. Primary spermatocyte showing a continuous network of chromatin threads, an unusual number of which have fused; some are still unpaired while others are in the process of fusing.

FIG. 10. Primary spermatocyte in process of degeneration in the "spireme stage."

FIG. 11. Primary spermatocyte in the late "spireme stage," showing signs of paired and pairing threads, paired and single chromosomes, and also signs of degeneration.

FIG. 12. Late prophase of primary spermatocyte showing univalent and bivalent chromosomes, and one trivalent chromosome. Some of the univalent components are still attached to each other at various angles. The accessory chromosome is very distinct.



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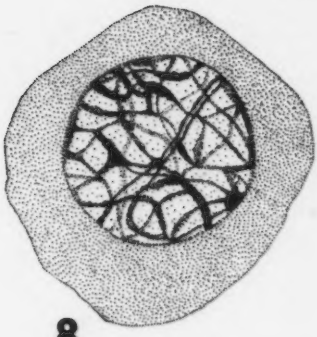

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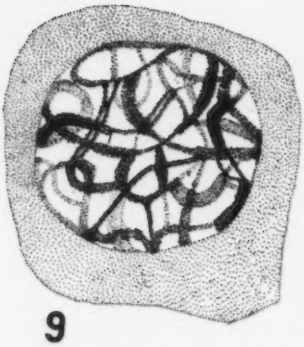

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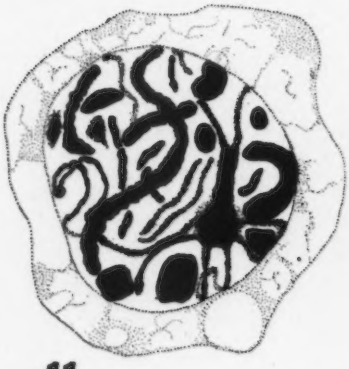

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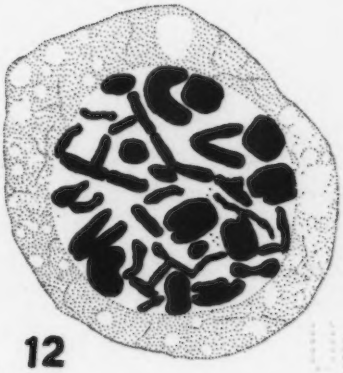

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PLATE III.

FIGS. 13–15. Late prophase of primary spermatocytes after the partial synapsis had taken place.

FIG. 13 shows forty chromosomes, about eleven of which appear to be bivalent.

FIG. 14 shows thirty-eight chromosomes about thirteen of which are bivalent. and Fig. 15 shows forty-three chromosomes about eight of which are bivalent Note the heart-shaped accessory in each of these cells.

FIG. 16. Polar view of a metaphase stage of a primary spermatocyte, showing twenty-eight chromosomes in the plate, and seventeen including the accessory are scattered in the cytoplasm.

FIGS. 17 AND 18. Degenerating primary spermatocytes in the metaphase stage showing many scattered chromosomes and the remains of the decayed cytoplasm congregated about the plate or large clump of chromosomes.

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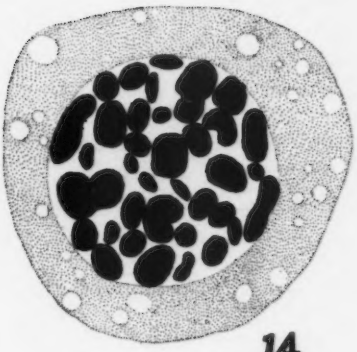

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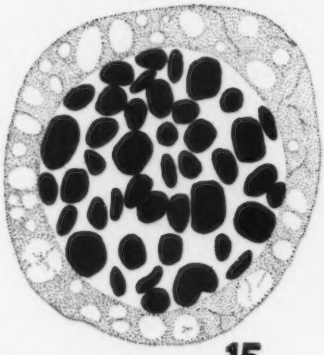

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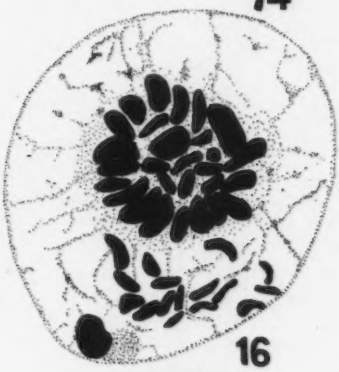

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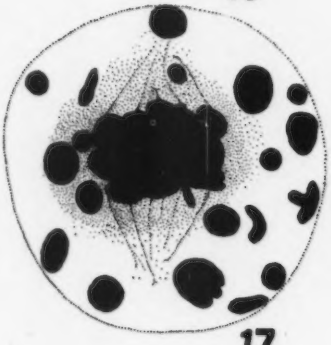

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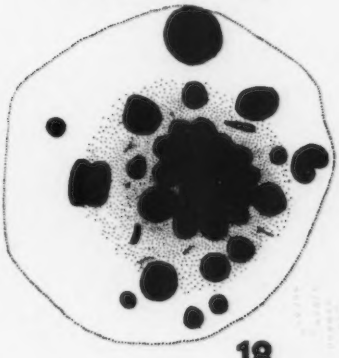

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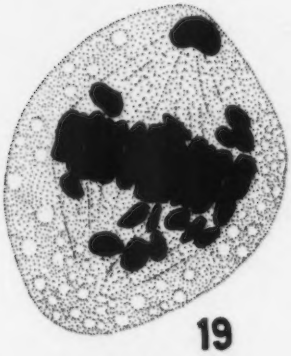
PLATE IV.

FIGS. 19–21. Freak types of primary spermatocytes in the metaphase stage showing most of the chromosomes in the equatorial plate and several scattered about on the spindle or in the cytoplasm.

FIG. 22. Primary spermatocyte showing an uncommon type of unequal division in which five large chromosomes have passed over to one place while about thirty-six remained in the original place.

FIG. 23. A somewhat degenerate metaphase stage of primary spermatocyte showing the expulsion of a bunch of chromosomes.

FIG. 24. A late telophase of a trinucleated primary spermatocyte. The nucleus on the outside of the cell wall was apparently formed from a group of chromosomes which were expelled by the cell.



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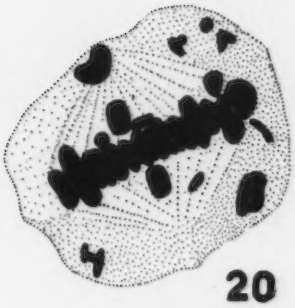

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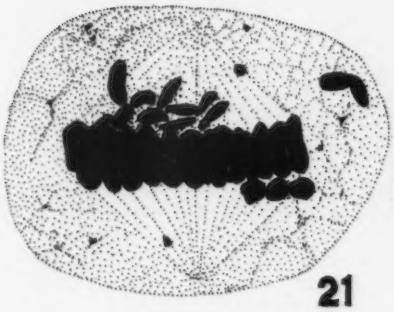

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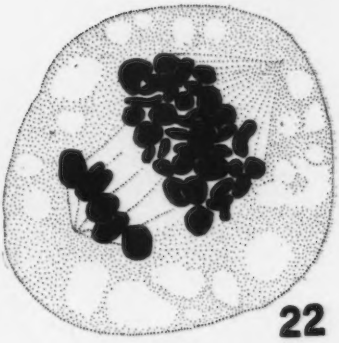

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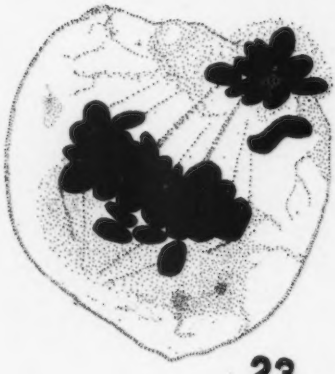

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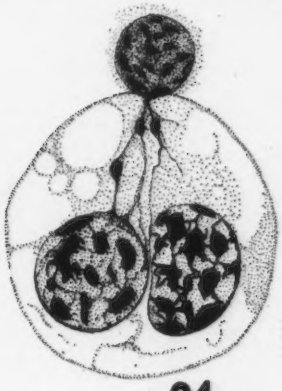

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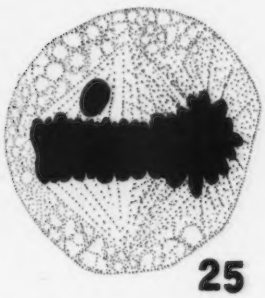
PLATE V.

FIGS. 25–30. Abnormal mitotic figures of primary spermatocytes (fully explained in the text).



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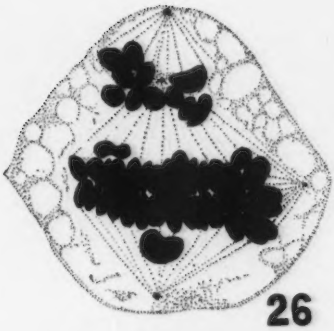

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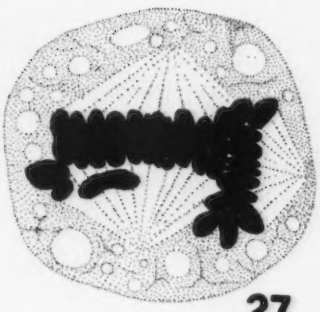

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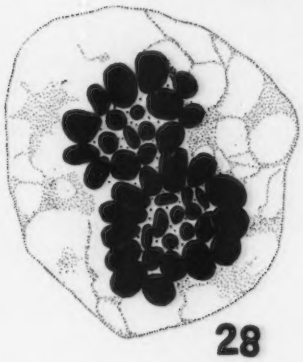

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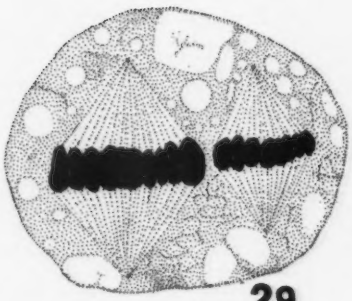

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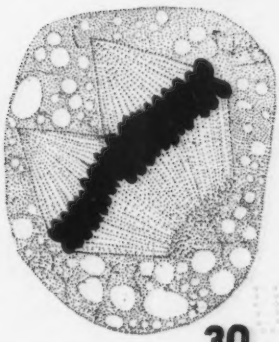

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PLATE VI.

FIGS. 31–36. Abnormal mitotic figures of primary spermatocytes (fully explained in the text).

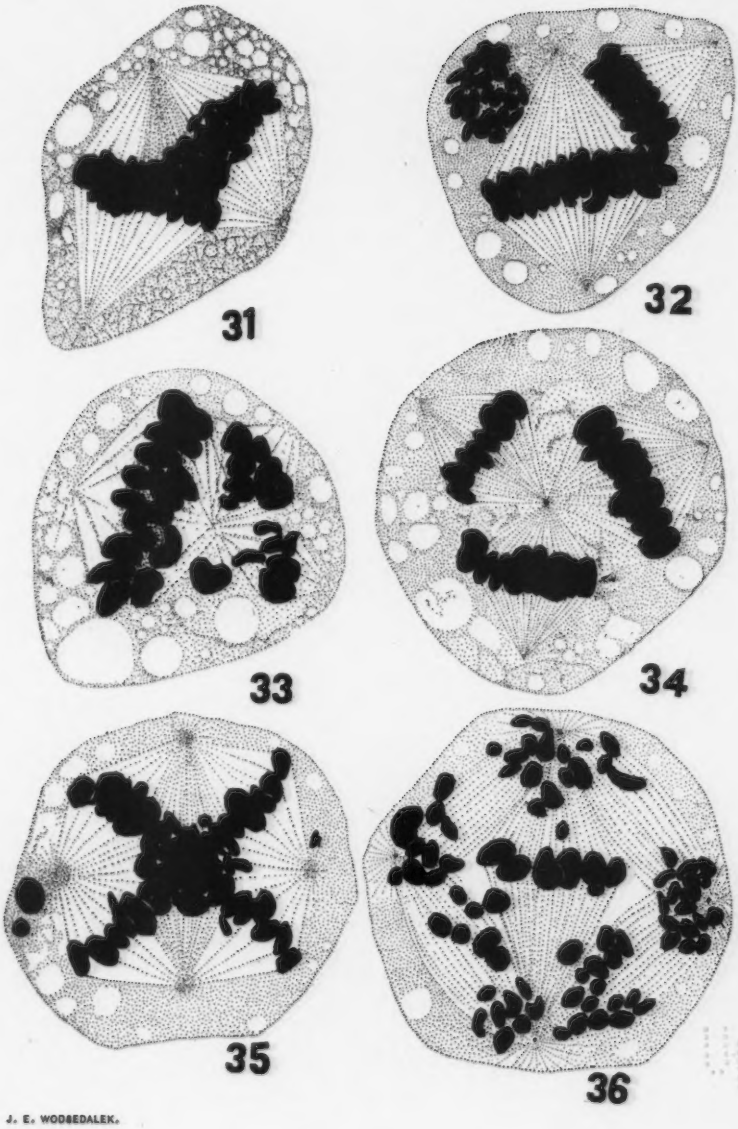


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PLATE VII.

FIG. 37. A giant cell with three spindles in the early anaphase stage.

FIG. 38. A giant cell with four large nuclei, each resembling the nucleus of the primary spermatocyte. (See Fig. 7.)

FIG. 39. A giant cell with a huge non-symmetrical quadripolar spindle containing over one hundred chromosomes. It is probably a spermatogonial cell which possessed two complete nuclei and the number of chromosomes in excess of one hundred and two may be due to the fact that many of the chromosomes have divided.

FIG. 40. A quadri-nucleated cell.

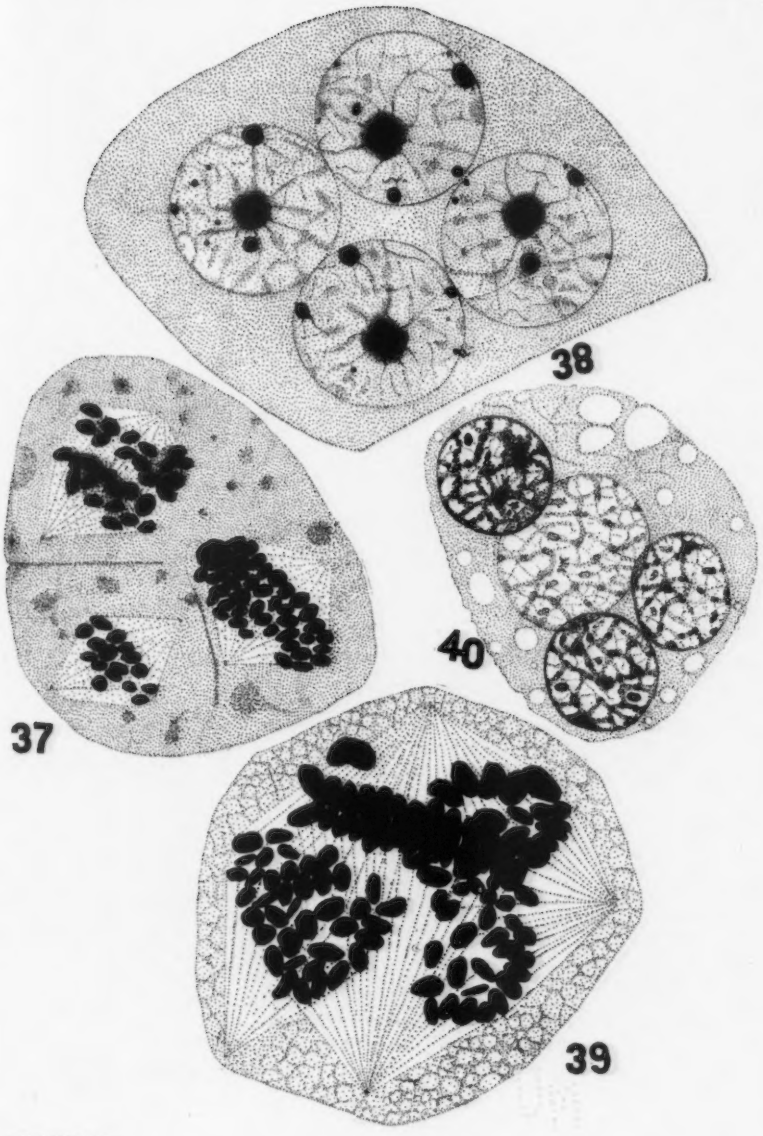


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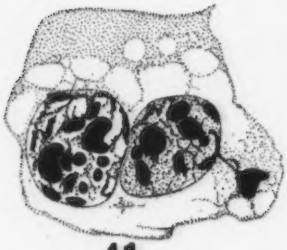
PLATE VIII.

FIG. 41. A bi-nucleated cell in process of degeneration showing a chromosome, possibly the accessory, which was left out in the cytoplasm.

FIG. 42. A cell with six well-defined nuclei.

FIG. 43. A degenerating cell with three nuclei.

FIGS. 44–46. Cells in process of degeneration (explained in the text).



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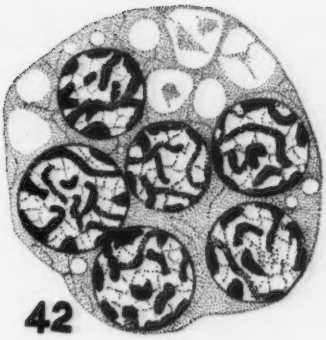

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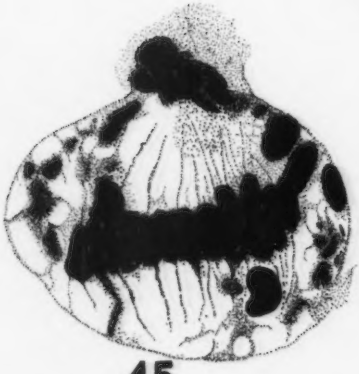

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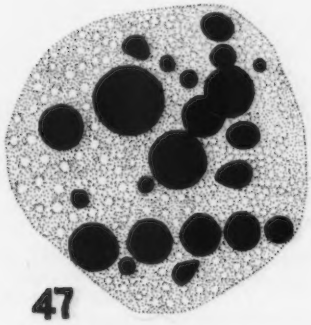
PLATE IX.

FIGS. 47–52. Various types of cells in process of degeneration (explained in the text).



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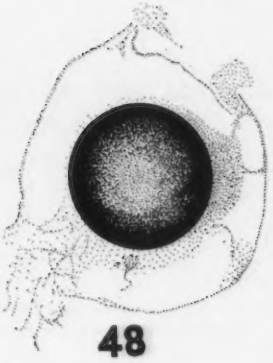

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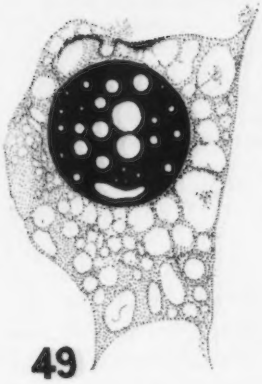

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# STUDIES ON THE GEOTROPISM OF THE MARINE SNAIL, LITTORINA LITTOREA.[1]

SAKYO KANDA.

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## I. INTRODUCTION.

In 1888, Loeb pointed out that "Die Schwerkraft der Erde, wenn sie senkrecht gegen die ventrale Seite der Schabe gerichtet ist, wirkt als Reiz, der dieselbe zu Bewegungen veranlasst" (8, p. 9). Since then, with modifications of his method, Davenport and Perkins (2) and Frandsen (3) have investigated the same problem on a slug, *Limax maximus*. The former drew the conclusions that, "the precision of orientation of the slug varies directly with the active component of gravity" (2, p. 105) and that "this tendency (to go either up or down) must be ascribed to some internal condition of the individuals, for it varies in different individuals and in the same individuals at different times" (2, p. 110). The latter reached rather different conclusions, thus: "The different geotactic response, on a glass plate, of different individuals is due mainly to two factors: (*a*) The quantity and quality of the slime secreted, which is a very important factor; (*b*) the relative proportions of the length of the

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Physiological Laboratory, University of Minnesota, Minneapolis, Minn.

anterior and posterior regions of the animal's body. All the conditions being the same, it is this factor which 'determines whether the head end will be directed up or down'" (3, p. 205).

The reactions of the marine forms of *Littorina littorea*, *L. rudis*, etc., to light and other influences have been studied by Mitsukuri (12), Bohn (1), Haseman (4), and Morse (14). Unfortunately, however, none of them has taken into consideration the response of the animals to gravity, although the effect of gravity upon *Littorina* and upon gastropods in general is remarkable and easily observed at the seashore.

Following the example of Frandsen and of Davenport and Perkins, an attempt was made by the writer with *Littorina littorea* to determine: (1) "What relation exists between a variation in the pressure of gravity and the precision of orientation?" and (2) What "determines whether the head end will be directed up or down?"

The experimental work was done in the physiological department of the Matine Biological Laboratory at Woods Hole, Mass., during the summers of 1912 and 1913. The results obtained in 1912 used in this paper are indicated by the date, "1912." The others were all secured in 1913.

## II. Material and Methods.

1. *Material.*—The animal used in all the experiments was a marine snail, *Littorina littorea*, which is numerous about Woods Hole. The snails were collected by the writer in the morning or afternoon, just before a new series of experiments was carried on, and were kept in a large glass dish in running sea-water during experiments. The size of the animal used was about 1.5 x 1.1 cm.[1] It was found that this was the more convenient size for experimental purposes, because the bigger, *i. e.*, older, ones retreated into and remained for a long time in their shells when handled. The younger animals are more active and quicker to respond to stimuli.

The same individuals could not be used throughout any series of experiments; since their movements became abnormal, due

[1] The laboratory collector, Mr. Gray, told me that the snails which I used were about one year old.

possibly to fatigue. The same individuals could be used only for four or five trials. Ten individuals, sometimes 11 or 12 (with anticipation of falls), were used for each trial of a series of experiments. As will be seen in the tables, however, very often less than 10 individuals were left on the support for observation, the rest having fallen down.

2. *Methods.*—Loeb's method (8) modified by Davenport and Perkins (2) and by Frandsen (3), of the different angles of inclination of a support, on which the animals are to be placed, was adopted with variations. The support was a plain glass plate about 22 x 19 cm., marked off on one side into squares of one sq. cm. each, whereby it could be readily determined how far the animals moved from their original places.

The support, or plate, was placed in an apparatus by which it could be held at any ten-degree angle between the horizontal and vertical (see Fig. 1).

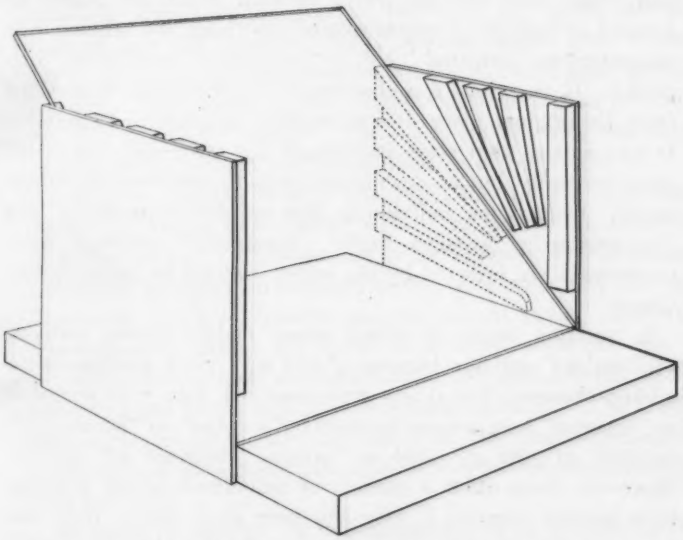

FIG. 1. *G. P.* = a plain glass plate. The rest of the apparatus is all wood.

The animals were placed on the unlined side of the plate, moistened and held upside down at an inclination, say 10° to

the horizontal, so that their heads were all directed upward. Meanwhile sea-water was poured, two or three times, over the animals and the plate surface to prevent their moving before the commencement of the experiment, and at the same time to get them to stick tightly to the surface. When the desired number of individuals had been so placed, the support was reversed, and placed at the desired angle in the holding apparatus, so that their heads were, now, directed downward. The animals being negative to gravity, this procedure was necessary in order to determine their movements of orientation in a desired time.

Experiments were conducted either in sea-water in a glass aquarium, or in air. To exclude the effect of light in either case, a square box painted black inside was employed to cover the whole arrangement described above.

After the desired time for a particular experiment, the cover-box was removed. Then the movements which the animals had made were recorded (with detailed notes) as nearly as possible as follows: A movement of 180° from the original was designated as "oriented"; 90° as "horizontal"; and 0° as "original." If an animal was observed to have crawled downward from the original place, it was recorded as positive to gravity. It was noticed that some individuals did not crawl downward quite vertically, but no discrimination as to these is made in the tables. Quite a few individuals, that crawled horizontally, are also arbitrarily classified under "horizontal," although such movements are believed by the writer to have no great significance.

A question might be raised about middle spaces between 180° and 90°, and also between 0° and 90°. Such positions were seldom observed; but if any were observed, they were recorded as "oriented" at a position between 90° and 180°; as "horizontal" between 90° and 45°; and as "original" between 45° and 0°. Moreover, since quite a number of individuals would possibly have become oriented if they had been given longer than one minute, it might have been better to describe them as "orienting" rather than "horizontal." The animals whose position was specified as "original" should not be interpreted as indifferent to gravity. They simply failed to respond to it during the time

when they were under experimentation. The time factor was important. This will be confirmed by the following preliminary experiment *A*. But for the more detailed study, the shortest possible time was adopted, to avoid fatiguing the animals.

In all the following tables, the signs indicate positive either to gravity or light by + and negative by −.

## III. Experiments.

### 1. *Preliminary Experiments with Gravity and Light.*

*Preliminary Experiment A.*—A tall beaker full of sea-water was placed upside down in a large dish filled with sea-water. Special care was taken to exclude air bubbles from the beaker. It was then supported on two pieces of glass-tubing about 4 mm. in diameter in the dish in order to let the sea-water within communicate freely with the outside at the bottom. By means of glass-tubing, sea-water was run into the dish so as to have fresh sea-water always at the bottom, but so as not to let any air bubbles into the beaker. Thirty selected snails were placed under the beaker, and between the two pieces of the glass-tubing, which was not high enough to permit the snails within to crawl out from under the beaker. The whole arrangement was then covered with the box already described, to exclude light.

About ten minutes later it was found that all the snails had crawled up the vertical (inside) wall of the beaker and gathered at or near its top. The sea-water would be expected to be fresher and better supplied with oxygen at the bottom than at the top. The snails, however, crawled upward just the same. This was repeated several times, but there was no exception to this rule.

Such results indicate that the upward movements of the snails are not caused by the lack of oxygen but by gravity. This is also fully supported by the fact that the snails crawl upward on moist rocks, or on a moist glass plate, in air where the amount of oxygen does not vary.

Moreover, in leaving the horizontal bottom of the dish to take positions on the vertical wall of the beaker, the snails must have oriented themselves against the pull of gravity. This orientation of the snails cannot be explained by the mechanical theory of geotropism. It is an active process, one of true response to

stimulation. Loeb's conclusion which was already referred to, is thus confirmed.

*Preliminary Experiment B.*—According to Bohn and Mitsukuri, *Littorina littorea* is negatively heliotropic. To test their results, a simple method of "righting" experiments was adopted as follows:

Ten selected individuals were placed dorsal side down on each of two glass plates. The one was covered with the box, and at the same time, the other was exposed to diffused daylight, both for five minutes. The two lots were alternately covered and exposed to daylight, after the animals had been refreshed in sea-water and again turned "on their backs." The results given in Table I. show that light considerably affects the "righting" response of the animals.

TABLE I.

THE "RIGHTING" OF SNAILS ON A HORIZONTAL GLASS PLATE IN DARKNESS AND DAYLIGHT FOR FIVE MINUTES (1912).

| No. of Trials. | No. of Animals. | Darkness. | | No. of Animals. | Daylight. | |
|---|---|---|---|---|---|---|
| | | Righted. | Not Righted. | | Righted. | Not Righted. |
| 1 | 10 | 4 | 6 | 10 | 3 | 7 |
| 1 | 10 | 8 | 2 | 10 | 2 | 8 |
| 1 | 10 | 6 | 4 | 10 | 3 | 7 |
| 1 | 10 | 5 | 5 | 10 | 2 | 8 |
| 1 | 10 | 6 | 4 | 10 | 3 | 7 |
| 1 | 10 | 6 | 4 | 10 | 2 | 8 |
| 1 | 10 | 4 | 6 | 10 | 4 | 6 |
| 1 | 10 | 4 | 6 | 10 | 2 | 8 |
| 1 | 10 | 5 | 5 | 10 | 3 | 7 |
| 1 | 10 | 4 | 6 | 10 | 2 | 8 |
| Total..10 | 100 | 52 or 52% | 48 or 48% | 100 | 26 or 26% | 74 or 74% |

In explanation of this "righting" reaction of the snails, it may not be out of place to refer to Loeb's analysis in the starfish. According to him, the "righting" of the starfish is a stereotropic phenomenon, but not geotropic (10, pp. 64–65). Recently Moore confirms this conclusion through experiments (13, p. 237), while Jennings seems to have been misled in this respect (5, pp. 120–148). The "righting" of the snails may also be a stereotropic phenomenon, though it makes no difference for the present purpose. In fact, however, as will be seen later, contact stimuli interfere with the snails' reaction to gravity. The main point in

regard to Preliminary Experiment *B* is that light is a factor in the behavior of these animals.

*Preliminary Experiment C.*—According to the "mechanical theory" of geotropism, an animal becomes oriented head up because of its heavier posterior region, or it orients itself with its head down, because of its heavier anterior region or head. An experiment was, therefore, made to determine which region of the snails, the anterior or the posterior, was heavier. This was simple. When an individual was placed on a glass plate in sea-water with its dorsal side down, it came out of the shell, and made an "effort" to "right." It was then carefully dropped and watched as it sank, before it retreated into the shell. Every one of them sank with its anterior region up, as expected. The was done also with those individuals which were positive to gravity at a certain angle of inclination. There was, however, no exception.

With the same point in mind, empty shells were tested. One of them was fixed by its ventral side on a small square cover glass, with as little glue as possible. At the three corners of the cover glass, fine threads were fastened. The center of gravity was found by suspension to be located somewhere in the posterior region. This was done with a number of shells and the same results were obtained in every case. These results, therefore, agree with the other observations, indicating that the posterior region of the snails is heavier than the anterior region.

From the above results, the writer is justified in concluding that the center of gravity of the snails is located in the posterior region; and a possible inference from this conclusion is that the posterior region of the snails has a greater specific gravity than the anterior region. This fact and inference may have a bearing in deciding whether the orientation of the animals against gravity is due to purely physical or mechanical pull. This question will be considered throughout all the following experiments.

### 2. *Experiments to Show the Relation between the Pressure of Gravity and the Precision of Orientation.*

*Experiment A.*—Davenport and Perkins asked and answered by experiment: "What relation exists between a variation in the

pressure of gravity and the precision of orientation of the slug?" (2, p. 100). An attempt was made by the writer to determine experimentally the same question for *Littorina littorea*. The experiments were conducted in sea-water. Light was excluded by means of the cover-box. The results given in Table II. show that the higher the angle the larger is the number showing *negative* geotropism, and the lower the angle the larger the number of (so-called) *positively* geotropic animals.

## TABLE II.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE IN SEA-WATER IN TOTAL DARKNESS.

At beginning of experiments each head pointing downward. Table shows results after one minute.

| Temp. of Sea-water. | Angles. | No. of Trials. | No. of Animals. | − Geotropism. | | | | + Geotropism. | | Did Not Move. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oriented. | | Horizontal. | | Crawled Downward. | | | |
| | | | | No. | %. | No. | %. | No. | %. | No. | %. |
| 18½°–21° C. | 90° | 35 | 300 | 300 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18½°–18½° C. | 78¾° | 22 | 200 | 200 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18½° C. | 67½° | 22 | 200 | 190 | 95 | 3 | 1.5 | 5 | 2.5 | 2 | 1 |
| 19°–19½° C. | 56¼° | 23 | 200 | 180 | 90 | 9 | 5.5 | 7 | 3.5 | 4 | 2 |
| 19¼°–19½° C. | 45° | 25 | 200 | 163 | 81.5 | 13 | 6.5 | 12 | 6 | 12 | 6 |
| 19½°–20° C. | 33¾° | 32 | 200 | 152 | 76 | 15 | 7.5 | 16 | 8 | 17 | 8.5 |
| 19½°–20° C. | 22½° | 33 | 200 | 130 | 65 | 22 | 11 | 23 | 11.5 | 25 | 12.5 |
| 20°–20¼° C. | 11¼° | 23 | 200 | 110 | 55 | 26 | 13 | 34 | 17 | 30 | 15 |
| Total..... | | 215 | 1,700 | 1,513 or 89% | | | | 97 or 5.7% | | 90 or 5.2% | |

As already pointed out, however, it is misleading to consider that the negative geotropism of the snails reaches zero on the horizontal surface of the earth. The force of gravity stimulates the snails, when it happens to pull along the line of the dorso-ventral axis of the animals, that is, on the horizontal surface, as the preliminary experiment *A* has shown. But the larger the angle the shorter the time required for orientation. This is shown in the "horizontal" column, when the number of the animals, most of which were "orienting" at the end of one minute, decreases as the angle of inclination to the vertical increases.

*Experiment B.*—It has been supposed that hydrostatic pressure affects the reaction of an animal to gravity. To decide this question, a series of experiments was made in air. Light was

excluded as usual. The results given in Table III. show an interesting difference compared with those of Table II., which must not be overlooked. The number in the negative geotropism column in Table III. is always higher than the corresponding one in Table II., and that in the positive column in the former is always lower than that in the latter. A possible explanation of these differences is found in the buoyancy effect of the water, which decreases the weight of the animals. In consequence, the pull of gravity is more effective on the animals in air. It would seem therefore that the pull of gravity on the animal *as a whole* must be one factor in its orientation.

## TABLE III.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE IN AIR IN TOTAL DARKNESS.

At beginning of experiments each head pointing downward. Table shows results after one minute.

| Temp. of Room. | Angles. | No. of Trials. | No. of Animals. | — Geotropism. | | | | + Geotrop. | | Original. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oriented. | | Horizontal. | | Crawled Downward. | | Did Not Move. | |
| | | | | No. | % | No. | % | No. | % | No. | % |
| 21¾°–22½° C. | 90° | 20 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25° C. | 78¾° | 14 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22½°–23° C. | 67½° | 15 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23° C. | 56¼° | 13 | 100 | 92 | 92 | 2 | 2 | 2 | 2 | 4 | 4 |
| 23½° C. | 45° | 13 | 100 | 86 | 86 | 6 | 6 | 6 | 6 | 2 | 2 |
| 21½° C. | 33¾° | 17 | 100 | 80 | 80 | 8 | 8 | 9 | 9 | 3 | 3 |
| 22¾°–23° C. | 22½° | 13 | 100 | 67 | 67 | 13 | 13 | 18 | 18 | 2 | 2 |
| 22° C. | 11¼° | 14 | 100 | 60 | 60 | 13 | 13 | 27 | 27 | 0 | 0 |
| Total..... | | 119 | 800 | 727 or 90.8% | | | | 62 or 7.7% | | 11 or 1.3% | |

The above fact reminds the writer of the "mechanical theory" (6, pp. 2–13) and, also, the "resistance or weight theory" (6, pp. 14–20) of geotropism in *Paramecium*. If the greater weight of snails in air causes the quicker orientation of the animals, and also if the posterior region of the animals is heavier than the anterior region (as has been proven to be the case), the theories above mentioned are favored to some extent. Nevertheless, no mechanical theory can satisfactorily explain why "positive geotropism" increases with decrease in the angle of inclination. This, if a real gravity response, must be explained on the basis of physiological conditions, as Loeb pointed out many years ago.

The question, however, arises: Is there any maximum or minimum of resistance or weight which makes the snails crawl either up or down? This question will be answered later.

Although differences exist between Tables II. and III., the results in the latter table in general show a fair uniformity with Table II. The hydrostatic pressure,[1] therefore, seems seldom, if ever, to affect the negative reaction of the animals to gravity. These two tables also show a fair agreement with the results obtained by Loeb (8, pp. 6–8), Davenport and Perkins (2, pp. 100–105), and Frandsen (3, p. 198), that is, a steady increase in negative geotropism with increase in the angle of inclination. Davenport and Perkins's view in this respect is fully confirmed (2, p. 105).

### 3. *What Determines Whether the Head End Will be Directed Up or Down?*

*Experiment A.*—Instead of the plain glass plate used for experiments hitherto, a ground-glass plate was employed for the following experiments, for two reasons: (1) Since the animals live along a rocky seashore, the plain glass plate may not be a proper support for them; and (2) the animals being positively stereotropic, it was thought well to test the effect of contact stimuli upon their geotropism. Light was excluded in these experiments as usual. In the results given in Table IV. it is interesting to note, as expected, a decided decrease in the number of negatively geotropic and an increase of "positively geotropic" individuals with decrease in the angle of inclination. Moreover, below the 45°, no individuals were in their original position.

The animals can stick better to the ground plate than they do to the plain glass. In consequence, they can resist the pull of gravity better on the former than they do on the latter. If so,

[1] On preparing Mr. Kanda's Ms. for publication it strikes me that the chief difference between the experiments shown in Tables II. and III. lies in the buoyancy rather than the pressure factor. Remembering that the air pressure must be added to that due to any depth of water, the difference in pressure in Table II. as contrasted with III. is almost negligible. But the weight of the animal in water is much less than in air. The slight difference between Tables II. and III. is to me strong evidence against the buoyancy theory as playing any large part. Mr. Kanda is in Japan and I cannot call his attention to these points, so I venture to add this footnote. —E. P. Lyon.

this serves to indicate that resistance plays a part, particularly in negative geotropism. The resistance theory, however, does not explain why the animals leave the horizontal surface on which they are placed and crawl upward on the vertical wall of a beaker (see preliminary experiment). This fact is unexplained by any mechanical or resistance theory.

## TABLE IV.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GROUND-GLASS PLATE IN AIR IN TOTAL DARKNESS.

At beginning of experiments each head pointing downward. Table shows results after one minute.

| Temp. of Room. | Angles. | No. of Trials. | No. of Animals. | − Geotropism. | | | | + Geotropism. | | Original. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oriented. | | Horizontal. | | Crawled Downward. | | Did Not Move. | |
| | | | | No. | % | No. | % | No. | % | No. | % |
| 22⅓°–22¾° C. | 90° | 14 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22° –22½° C. | 78¾° | 16 | 100 | 96 | 96 | 0 | 0 | 2 | 2 | 2 | 2 |
| 22¾°–23° C. | 67½° | 13 | 100 | 82 | 82 | 6 | 6 | 10 | 10 | 2 | 2 |
| 23° –23½° C. | 56¼° | 13 | 100 | 71 | 71 | 10 | 10 | 16 | 16 | 3 | 3 |
| 27° C. | 45° | 12 | 100 | 63 | 63 | 12 | 12 | 25 | 25 | 0 | 0 |
| 25¼° C. | 33¾° | 11 | 100 | 53 | 53 | 13 | 13 | 34 | 34 | 0 | 0 |
| 24¾°–25½° C. | 22½° | 10 | 100 | 39 | 39 | 20 | 20 | 41 | 41 | 0 | 0 |
| 24¾°–25¾° C. | 11¼° | 10 | 100 | 23 | 23 | 22 | 22 | 55 | 55 | 0 | 0 |
| Total..... | | 99 | 800 | 610 or 76.2% | | | | 183 or 22.8% | | 7 or .8% | |

*Experiment B.*—A few experiments were made to determine the effect of a dry surface at the angle of 90°. The animals experimented on were carefully dried on filter paper. They were placed on a dry smooth glass plate with their heads directed *upward*. Light was excluded and the animals examined after two minutes. The movements of the animals on the plate were readily seen by the tracks of the mucus secreted by them.

Of 60 animals used 31 dropped off, 6 or 15 per cent. of the remainder oriented and crawled down, 31 or 79 per cent. crawled upward and one crawled horizontally. The striking fact is the number of individuals that oriented *positively* and crawled down. This evidence goes to show that the animals tend to become positive on a dry surface, which would evidently serve as a protective reaction when they are left on rocks by a retreating tide.

The movements of the animals on the dry plate were regular

in some cases and irregular in others. The figures given are, therefore, somewhat arbitrary. To avoid this inaccuracy, a few typical examples of movement are shown in figures as follows:

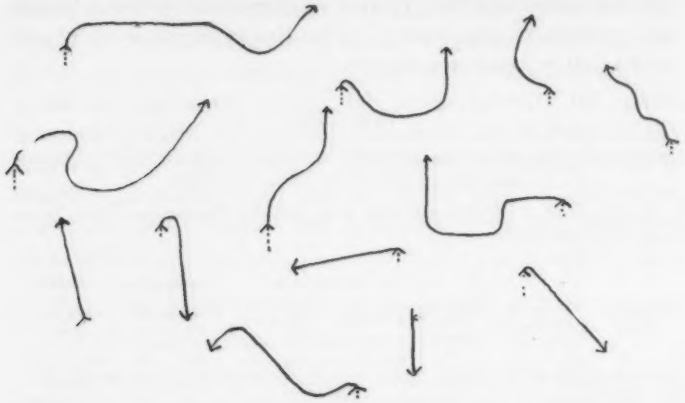

FIG. 2.

The arrows in the figure indicate the movements during the experiment, and the dots the movements of the animals after being placed on the plate but before the commencement of the experiment, *i. e.*, before the plate was covered by the dark box.

One thing is clear. Some of the animals on the dry glass plate and also those placed on a dry wooden plate, in an experiment the results of which will be seen shortly after this, oriented themselves and crawled downward, in spite of the mechanical difficulties due to their shells.

*Experiment C.*—In the next series, a dry wooden plate was used instead of the dry glass plate, in the same manner as in the above experiment. Of 60 animals used 24 dropped off, 19 or 52 per cent. of the remainder oriented and crawled downward, 6 or 16 per cent. crawled up and 4 crawled horizontally. Positive instead of negative geotropism was dominant, even though the experiments were conducted at the angle of 90°.

*Experiment D.*—The following series was of the same nature, except that the animals were placed with their heads down, and the time of experimentation was limited to one minute. Of 60 individuals used 33 dropped, 21 or 77 per cent. of those remaining

on the plate crawled downward; only 2 oriented negatively and these did not crawl upward; 4 failed to move. As the animals were not compelled to orient themselves in order to move down, the highest percentage of positive geotropism (21 individuals out of 27 or 77 per cent.) was obtained in this case.

That the character of the surface played a part is evident. But some explanation is necessary. When the cover-box was removed, nine individuals which had crawled down as shown by the mucus were found with the head end of their shells directed vertically, or with slight inclination to the vertical, *i. e.*, their shells mechanically "oriented" *upward* by the "greater pull of gravity" on the "heavier posterior region." Those individuals probably could not resist the pull of gravity and keep their shells from falling by their weight, while others could. Four individuals crawled downward not quite vertically (on a diagonal line between vertical and horizontal). It may be possible to explain this as a movement which was resultant of the pull of gravity on their shells and on themselves,—the resultant of a passive tendency to orientation and an internal response to gravitation.

From the above observations, one may explain the habitat of the snails, "which usually live on rocky surfaces which lie between the high-tide mark and one foot below the low-tide mark." The limitation of upward movement is due chiefly to the exhaustion of moisture carried by their "foot" from the sea. They seldom, if ever, crawl on dry rocks much higher than high-tide mark, though they do crawl on rocks clear out of sea-water. They seem to stop crawling when the moisture, which they carry with themselves, is exhausted. This agrees with Mitsukuri's and Haseman's observations.

It is not thinkable that either the dry glass plate, or the dry wooden plate makes it easier for the snails to become oriented and to crawl downward, than the ground-glass plate did. Nevertheless on the former surfaces many animals oriented themselves and crawled downward against the resistance of the heavier weight of their posterior region. If this is true, then the upward and downward movements of the snails can not be explained either by the resistance factor or the weight factor or by the both.

One thing is, of course, possible, that "the quantity and quality

of the slime secreted" makes it easier for the animals to crawl downward or upward, as Frandsen thinks in the case of slugs (3, p. 205). The question, however, arises: Why, then, did not the snails crawl upward instead of downward on the the dry glass and wooden plates?

In this respect, Parker and Parshley offer a suggestive explanation in the case of earthworms: "In the earthworm, the response to dryness . . . is apparently the selective extraction of water from the peripheral protoplasm of the worm, a process which is favored by capillarity of dry surfaces over which the worm begins to creep and is probably dependent chiefly upon evaporation from the surface of the worm itself. Under such circumstances, the materials in the peripheral protoplasm of the prostomium must become concentrated and probably initiates stimulation by undergoing some such change as partial coagulation" (15, p. 363).

If so, the explanation must be looked for in physiological conditions, that is, internal, rather than external mechanical factors. As Parker and Parshley explain, the dryness of the surface may cause the partial coagulation of the materials in the peripheral protoplasm of the "foot" as well as of the anterior geo-sensitive region of the snails, which may also result in surface-tension changes and contraction of "muscle elements," and cause the motion of displacement or coalescence of the fluid materials in the protoplasm of the muscle-fibers and internal cells (as Lillie thinks; 7, pp. 252–255), which possess different specific gravities. Under such circumstances, we may assume a disturbance in the peripheral as well as internal cells, especially perhaps in the cells of the geo-sensitive region or the geotropic organs, statocysts, which disturbance may be assumed to stimulate and cause the response of the animals. Whether the animal will go up or down will depend on the other stimuli besides gravity, such as character of surface and degree of dryness which accompany the stimulation of gravity itself. This view quite agrees with Loeb's (9) and Lyon's (11 or 6, pp. 20–21).

### 4. *The Effects of Light and Surface-film of Sea-water.*

*Experiment A.*—Experiments were conducted in the aquarium already described, which was closely surrounded by a four-fold black cloth, all over the sides and bottom. The snails were placed on the glass plate about 2.5 cm. below the surface film of sea-water. Part of the glass plate extended above the surface. By this arrangement, it was possible at the same time to test Haseman's conclusion that the upward and downward movements of the animals "are not due directly to either geotropism or phototaxis, but to the action of the film of water" (4, p. 113). The results are given in Table V.

TABLE V.

GEOTROPISM OF SNAILS IN AND OUT OF SEA-WATER IN DIFFUSE DAYLIGHT AT DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE.

At beginning of experiments each head placed pointing *downward*. Table shows results after one minute.

| Temp. of Sea-water | Angles. | No. of Trials. | No. of Animals. | — Geotropism and + Heliotropism. | | | | | | — Heliotropism and + Geotropism. | | | | Crawling Horizontally in Sea-water. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Crawling up in Air. | | Crawling up in Sea-water. | | At Surface-Film. | | Crawling Downward. | | Crawling Diagonally Downward. | | | |
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| 21½°–19½° C. | 90° | 15 | 50 | 14 | 28 | 21 | 42 | 9 | 18 | 0 | 0 | 0 | 0 | 6 | 12 |
| 20½° C. | 45° | 14 | 50 | 7 | 14 | 17 | 34 | 3 | 6 | 7 | 14 | 12 | 24 | 4 | 8 |
| 20½° C. | 11¼° | 10 | 50 | 0 | 0 | 2 | 4 | 0 | 0 | 15 | 30 | 27 | 54 | 6 | 12 |
| Total..... | | 39 | 150 | 73 or 48.6% | | | | | | 61 or 40.6% | | | | 16 or 10.6% | |

At the angle of 90°, none were positively geotropic and only six individuals out of 50 crawled horizontally in sea-water (possibly due to the effect of light). At the angles of 45° and 11¾°, the number showing "positive geotropism" increased, as would be expected. At the end of one minute, 21 individuals out of 150 (14 at 90° and 7 at 45°), as shown in the first column of negative geotropism, crawled upward clear through the surface-film of sea-water into the air. 12 individuals (9 at 90° and 3 at 45°), as shown in the last column of the same, crawled upward until they reached the surface-film of sea-water, and then

crawled horizontally. Was this due "to the action of the film of water," as Haseman claims? The question will be answered after consideration of a few other experiments.

*Experiment B.*—The next series of experiments was of the same nature as the above, but the animals were placed with their heads upward instead of downward at the beginning. The results given in Table VI. show that the starting position, that is, placing the animal head downward or upward, at the beginning of the experiment, makes a difference in the result.

## TABLE VI.

GEOTROPISM OF SNAILS IN AND OUT OF SEA-WATER IN DIFFUSE DAYLIGHT AT DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE.

At beginning of experiments each head placed pointing *upward*. Table shows results after one minute.

| Temp. of Sea-water. | Angles. | No. of Trials. | No. of Animals. | − Geotropism and + Heliotropism. | | | | | | | | + Geotropism and − Heliotropism. | | | | Crawling Horizontally in Sea-water. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Crawling Up in Air (No Hesitation at Surface). | | Crawling Up in Sea-water (Not yet at Surface). | | Hesitated at Surface then Crawled Through into Air. | | Hesitated at Surface and Crawled Horizontally. | | Crawling Downward. | | Crawling Diag. Down. | | | |
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| 19½° C. | 90° | 14 | 50 | 23 | 46 | 0 | 0 | 6 | 12 | 11 | 22 | 0 | 0 | 0 | 0 | 10 | 20 |
| 21¼° C. | 45° | 13 | 50 | 19 | 38 | 12 | 24 | 3 | 6 | 5 | 10 | 1 | 2 | 8 | 16 | 2 | 4 |
| 21½° C. | 11¼° | 10 | 50 | 3 | 6 | 24 | 48 | 0 | 0 | 0 | 0 | 8 | 16 | 11 | 22 | 4 | 8 |
| Total..... | | 37 | 150 | 106 or 70.6% | | | | | | | | 28 or 18.6% | | | | 16 or 10.6% | |

Here, again, eleven individuals at the angle of 90° and five at 45° crawled horizontally beneath the surface-film of sea-water. Besides these, six individuals at the angle of 90° and three at 45° "hesitated" at the surface-film when they reached it, and then crawled upward through the film. This constitutes a puzzle for the surface-film theory. But let us see further.

*Experiment C.*—Similar experiments were made in direct sunlight. The glass plate in the aquarium was placed at 45°, which made it parallel to the rays of sunlight as nearly as possible. The outside and bottom of the cylindrical glass aquarium were surrounded by black cloth as already stated; and two sections, *a* and *b* (see Fig. 3), separated within by the glass plate, were

covered above so that the sunlight penetrated parallel to the glass plate and through the open portion, *c*. So arranged, the intensity and direction of the rays of the sunlight had to affect the reaction of the snails to gravity.

Since the animals were negatively heliotropic, it was expected that most of them would crawl downward in this arrangement. Their heads, therefore, were placed upward at beginning, so that definite movements of orientation could be observed. The

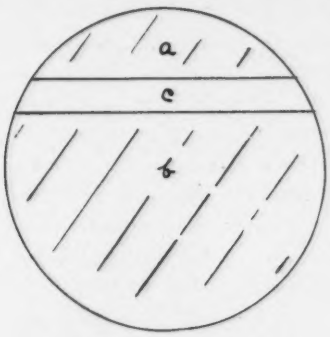


FIG. 3.

results after one minute were as follows: Of 50 animals in 15 trials, 5 or 10 per cent. had crawled upward, all diagonally; 37 or 74 per cent. had crawled downward, 22 of them being well oriented and 15 diagonally; 8 or 16 per cent. crawled horizontally and consequently across the lines of direction both of light and of the effective component of gravity.

The predominance of downward movement was no doubt due to the intensity and direction of the sunlight. This point becomes clearer in the next experiments, but it is clear that the surface-film has nothing to do with such reactions.

*Experiment D.*—With the same point in mind, other experiments were made in a similar way, but the animals were placed about 1.5 cm. above the surface of the sea-water. In this case 29 individuals out of 50 oriented themselves downward, and crawled in that direction through the surface-film of sea-water into it. Three crawled horizontally at the surface-film instead of going into the sea-water. 18 or 36 per cent. crawled upward.

### 5. *The Effect of the Surface-film of Sea-water.*

The last of this series was planned in a little different way from the above, entirely excluding light.

As a preliminary test, the snails were placed on the glass plate at the angle of 56¼° in the aquarium, which was half filled with sea-water. When those crawling upward reached the surface-film of sea-water and "hesitated," as Haseman calls it, beneath the film, the whole arrangement was covered with the dark box. Ten seconds later, the box was removed for observation. Nearly all that had "hesitated" at the film, were found to have crawled upward *through* the film.[1] Certain individuals at that time had already crawled upward as high as 3 cm. from the film, some 2 cm., and others were just above the film. This experiment was repeated several times and these results were readily demonstrable. Judging from the results, the animals did not seem to have "hesitated" long, after the light was excluded. The "hesitation" of the snails at the surface of sea-water seems to the writer to be due chiefly to the effect of light instead of to action of the surface-film of sea-water. This point also becomes evident after further consideration.

Quantitative experiments were conducted as indicated above. The results are tabulated in Table VII. and show rather complicated conditions.

## TABLE VII.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE IN AND OUT OF SEA-WATER IN TOTAL DARKNESS.

At beginning of experiment each head placed pointing *downward*. Table shows results after one minute and after another half minute.

| Temperature of Sea-water. | Angles. | No. of Trials. | No. of Animals. | − Geotropism | | | | | | | | | + Geotropism. | Crawled Horizontally in Sea-water. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *A.* After One Minute. | | | | | | *B.* After Another Half-minute. | | | | |
| | | | | Clear Out of Sea-water. | | Stopped Just Under Surface-film. | | Crawling up in Sea-water. | | Clear out of Sea-water. | Underneath Surface-film. | Crawling up in Sea-water. | After 1 Min. | After 1 Min. |
| | | | | No. | % | No. | % | No. | % | No. | No. | No. | No. | No. |
| 22° C. | 90° | 12 | 50 | 26 | 52 | 18 | 36 | 6 | 12 | 18 | 4 | 4 | 9 | 0 |
| 21½° C. | 45° | 14 | 50 | 23 | 46 | 11 | 22 | 6 | 12 | 14 | 2 | 1 | 3 | 5 |
| 21½° C. | 11¼° | 11 | 50 | 1 | 2 | 13 | 26 | 5 | 10 | 11 | 8 | 5 | 21 | 10 |
| Total..... | | 37 | 150 | 109 or 72% | | | | | | 67 | | | 24 | 15 |

[1] They change their "minds" very rapidly!

"*B*" covers the activity of those animals which were still below the surface at the end of one minute.

After the first minute, twenty-six individuals at the angle of 90°, twenty-three at 45°, and one at 11¼° were found to have crawled upward through the surface-film of sea-water. Eighteen individuals at the angle of 90°, eleven at 45° and thirteen at 11¾° were just beneath the film of sea-water. And after another half-minute, sixteen individuals at the angle of 90° out of the eighteen, which were beneath the film of sea-water at the end of one minute, nine at 45° out of the eleven, ten at 11¾° out of the thirteen, and one at the same angle which was crawling horizontally at the end of one minute, had crawled upward *through* the film of sea-water. This is significant. At the time of second observation, also, at the angle of 90° two individuals out of six, which were crawling upward *in* sea-water at the end of one minute, at 45° five out of six, and at 11¾° one out of ten which were crawling horizontally at the end of one minute, had crawled upward *through* the film. But at the angle of 90° two individuals out of eighteen which were beneath the film at the end of the first period, at 45° two out of eleven, and at 11¾° three out of thirteen, that is, only seven individuals altogether out of 150, *still remained* beneath the film even at the end of the second period. This is decidedly contrary to the surface-film theory. And at the angle of 90° two individuals out of six, which were crawling upward in sea-water at the end of the first period, at 11¾° four out of five, and at the same angle one which crawled downward in the first period, were beneath the film at the end of the second period.

Referring again to the observation of Haseman that "during a falling tide, some snails are crawling down beneath the surface, some with the surface, and some above the surface" (4, p. 120), and which Haseman claims is "due to the action of the film of water," but not "to either geotropism or phototaxis," the writer is inclined to draw the conclusion based on the results of the series of the experiments above, that the movements of the snails in question are not due, directly, if at all, "to the action of the film of water," but to geotropism and heliotropism. In nature, especially in the daytime when the sun is shining, a considerable part would be played by heliotropism, as Mitsukuri already observed.

Furthermore, in the above experiment, even the seven individuals, that still remained beneath the film at the end of the second period, and whose failure to penetrate the film seems to favor the surface-film action theory, may be considered in a different way. The effect of light having been excluded, their failure to emerge might be due to the susceptibility of these individuals to buoyancy when they had to crawl out of sea-water. This must be taken into consideration as has been shown. It might also in combination with the effect of light explain the behavior of those animals that crawled horizontally beneath the film, or that "hesitated" there, as shown in a number of the preceding tables. Whatever may be the explanation and even if one attributes the slight hesitation to the film itself, it is evident that it has very little effect in determining the total behavior of the animals.

### 6. *The Effect of Chemicals.*

Besides the above experiments, attempts were made to control the negative geotropism of the snails by chemicals, especially by salts and acids. But all were unsuccessful, except possibly an alcohol experiment. Five snails were placed in a finger bowl containing 100 or 150 c.c. sea-water, to which about 10 c.c. of 95 per cent. alcohol were added without stirring. The finger bowl was shaded. The animals crawled upward on the vertical wall of the bowl, but as soon as they reached the upper layers they turned round, and crawled downward. The negative geotropism was thus apparently reversed. But if the alcohol and sea-water were well mixed by stirring, the reversal was not definite and the experiment was not followed further.

## IV. Discussion.

That *Littorina littorea* is negatively heliotropic was first shown by Bohn (1), and this fact has been confirmed by the present writer. Morse, however, has published puzzling conclusions from his observations on this species. He states: "During the days of June, they were, as a rule, ***negatively*** phototactic, and as ***night*** approached, they became ***positively*** phototactic. However after July 18, the preponderance of ***positive phototataxis during the day*** was very noticeable. This period of transition corre-

sponded to the time of change from spring to neap tide, during which the specimens out *on the rocks* were exhibiting a corresponding change in phototaxis, for the water did not reach them; their behavior tallied with the description of Mitsukuri, who showed that when desiccated, periwinkles became positively phototactic, and when wet, turned negatively phototactic" (14, p. 145).[1]

Unfortunately, Morse has given no description of his methods of experimentation. But, judging from his statements, he has entirely overlooked the effect of gravity on the animal, as Mitsukuri did. Was not the supposed "positive phototaxis" "after July 18" really an effect of gravity? *Littorina littorea* does not, in the writer's opinion, crawl upward "on the rocks" on account of positive heliotropism, but on account of negative geotropism and *in spite of* negative heliotropism, which is the unmistakable reaction of the animal to light. Negative heliotropism in the animal is demonstrable even at "night," if the experiment is conducted on a horizontal surface, and if there is any source of light present. The writer believes that Morse's statement that "as night approached, they became positively phototactic" is incorrect. What really happens is this: As the light stimulus diminishes, the gravity stimulus becomes preponderant and the animals are controlled by their negative geotropism.

Frandsen, as already stated, proposes two factors for the determination of geotropism in the slug. The second will be considered first. "*All the conditions being the same*,"[1] he says, "it is this factor (the relative proportions of the length of the anterior and the posterior regions of the animal's body) which 'determines whether the head end will be directed up or down.' If the ratio of the length of anterior to posterior region of body is 2 : 3, or more, and the mucus is of good quality and sufficient quantity, the slug will be positively geotactic. If the ratio is 3 : 5, or less, the animal will usually migrate upward, and the nearer the ratio approaches 1 : 2 the more apt is the slug to respond negatively. . . . All slugs have a natural tendency to move towards the earth. This tendency is masked in the animals which are negatively geotactic on a glass plate by the greater pull of gravity

[1] Italics not in the original.

on the disproportionately larger and heavier posterior region of the animal" (3, p. 205).

Judging from these statements, the center of gravity must lie somewhere in the posterior region of the animal's body. In other words, the posterior region is *heavier* than the anterior. According to Frandsen, the negative geotropism of the animal "on an inclined glass plate," however, is not due to the heaviness of the posterior region, but to "the relative proportions of the length of the anterior and the posterior regions of the animal's body." In short, Frandsen's idea may be expressed thus: The longer (and heavier?) posterior region being pulled by the constant force of gravity, the slug becomes positive to it at the minimal resistance in favor of the ratio above mentioned; on the other hand, it becomes negative at the maximal in disfavor of the ratio. In the latter sense, the "resistance theory," of course, approaches the "mechanical theory."

As pointed out before, this seems to be fairly supported by the results obtained by the writer, which were given in Tables II., III. and IV. This explanation is, however, focused to a limited group of facts; because the snail becomes negative to gravity on the nearly horizontal surface of a glass plate, where the minimal resistance should be expected. This fact is opposed to Frandsen's conclusion. Furthermore, as has already been shown, many snails on the dry wooden plate at the angle of 90° of inclination oriented themselves downward and crawled in that direction, even though in so doing, they met a great mechanical difficulty on account of the heavier posterior region. If the mechanical theory of Frandsen is true, they were under the most favorable conditions for crawling upward instead of downward. This was not, however, the case. Was this because of the first factor of Frandsen, that is, "the quantity and quality of the slime secreted" (3, p. 205). The second factor in question is by no means separable from the first. Both go together. To make Frandsen's statement clearer, therefore, it may be expressed as follows: "The relative proportions of the length of the anterior and the posterior regions of the animal's body" "being the same, it is this factor," that is, "the quantity and quality of the slime secreted," "which determines whether the head end will be directed

up or down." It is fair to examine the table (V.) which is given by Frandsen based on his results. Take the animal No. "13," on which the series of observations, "*a*" and "*b*" were made. The condition of the animal was "active" in series "*a*" and also in series "*b*," that is, it was under the "same" condition. Being the *same* individual, theoretically there was no difference in the "ratio." Nevertheless, Frandsen obtained *different* results in "*a*" from "*b*." Moreover, he shows *different* results in the *same* individual under the *same* condition and in the *same* series of observations, "*a*" or "*b*." This is more striking in the animal No. "23," on which the series of observations, "*a*," "*b*," "*c*" and "*d*," were made under the *same* condition, "good." But he obtained 64.2 per cent. of negative geotropism in series "*a*," 62.5 per cent. in the "*b*," 100 per cent. in the "*c*," and 100 per cent. in the "*d*." In other words, the *same* individual under the *same* condition was, at least, 35.7 per cent. positive in "*a*," 37.4 per cent. in "*b*," and 0 per cent. in "*c*" and "*d*." This kind of variation is also found in the animals Nos. "8," "24," "25" and "27." If so, "all the conditions being the same," it is not entirely the first factor, nor the second, "which 'determines whether the head end will be directed up or down,'" but there must be something else besides these two factors, which makes the response variable. Frandsen does not seem, therefore, to be justified even by his experimental data in his conclusions. And strictly speaking, the so-called mechanical theory of geotropism seems to the writer to be misleading in any case, because it is not a living response to stimulus but a purely mechanical one which would be seen as well in the dead organism, if it could be moved. This is no tropism at all.

In this respect, therefore, the writer is inclined to take Davenport and Perkins's conclusion into consideration, as, at least, one of the factors, that is, "some internal condition of the individual." Without this "internal," or physiological, factor, it is difficult to explain why geotropism varies in the different individuals and also in the same individual at the different angles of inclination of the same support and the same angle of inclination of different supports.

Haseman's conclusions demand close consideration on several

points. Haseman states: "Even more interesting is the fact that when, with a vertical surface, a stone, upon which snails were crawling at random, was raised out of the sea, the snails always followed the vanishing film of water even when the vertical surface was rotated through an angle of 180°. In this case, the rotation of the vertical surface would reverse the direction of motion of the film of water and the snails would at once turn around and follow it. But if most of the water was previously removed from the surface of the stone, in order that the film might entirely disappear before the snails (which were crawling downward in the direction of the vanishing film) had reached the lower surface and if, as the film was drying up, the vertical surface was rotated through an angle of 180°, the snails continued to crawl for some time in the direction in which they had started. In other words, the snails crawled upward instead of downward. They continued to crawl thus until the rough surface, food and moisture either deflected or stopped their movements. In the above experiment, the mere turning of the moist but filmless surface through an angle of 180° does not seem adequate to reverse at once the reaction to gravity and light, if either of these have a direct influence on the rhythmical movements of *Littorina*" (4, p. 116).

Certainly this is not a simple matter. In the first phase, Haseman does not state, where "the above experiment" was conducted, what condition of sunlight or diffuse daylight, there was, and if the experiment was in sunlight, in what direction the rays were falling and so on. His description as well as experiment is not at all accurate. Judging from the above description, however, he seems to have conducted the experiment "in nature," and so in sunlight. If so and if the sun were fairly above, it is small wonder that "when the vertical surface was rotated through an angle of 180°," thus rotating the motion of the film of water, "the snails would at once turn around and follow it," since the snails are negative to light, as has been shown. In the case of Haseman where the film was reversed, in which "the vertical surface was rotated through an angle of 180°," and "the snails continued to crawl for some time in the direction (upward) in which they had started," another explanation is possible. The

snails being negatively geotropic, the direction and intensity of sunlight were not at the time of this experiment presumably in favor of the reversal of negative geotropism. Therefore, "the snails continued to crawl" "upward instead of downward." This is, of course, reasoned upon the results which were obtained by the writer. At any rate, Haseman seems not to have checked the counteracting forces of gravity and light and consequently all his experiments are unreliable.

One afternoon from four to six o'clock, Dr. Irving A. Field and the writer made special observations of tidal (rising) influence upon the snails at the south shore of Ram Island. The animals were found in large numbers covering a pair of long square beams which formed an inclined railway. These beams presented both vertical and sloping surfaces. In this vicinity there were also rocks and stones of various shapes, their surfaces sloping at many different angles on which numerous snails—oriented with their heads upward—were exposed in *dim* sunlight. When the tide rose higher and higher with no waves and reached to the areas where the animals had been exposed so long that their outer surfaces were completely dried, nearly all of them, if not the entire number, gradually turned head downward and crawled in that direction; some of them moved downward several inches from the original spots, while some others moved about "at random" as if they were seeking food; and still others turned downward when the surface-film of sea-water came in contact with them, while many did so after the surface-film had passed over them to the extent of 0.5 or 1 cm., more or less. If the snails follow "the direction of motion of the film of water," and also, if light has no influence on the rhythmical movements of *Littorina*, as Haseman claims, why did not the snails "crawl upward instead of downward"? Thus considered, it becomes evident that Haseman's observation, or experiment, was not accurate, while Mitsukuri's is, in this respect, confirmed by the writer.

It is very strange to note that Haseman has no notion of the influence of gravity on the snails, although he has observed phenomena which would naturally remind one of it. "When individuals are left high and dry on vertical surfaces during low

tide," he says, "they come to rest 'directed upward,' *i. e.*, with their heads toward the sky. This is true for all sides of the stones and is obviously due to the shape of the apertures of the shells which makes it far easier for exposed individuals to cling thus to vertical surfaces" (4, p. 117). How does he know that "the shape of the aperture of the shells" makes it easier to cling to vertical surfaces? Is it not rather true that the natural tendency of negative geotropism of the snails, favored by the *heavier* posterior region of the shells instead of the shape of the aperture, "makes it far easier for exposed individuals to cling thus to vertical surfaces"? This sounds more reasonable and nearer to the fact than Haseman's supposition.

## V. Summary and Conclusions.

1. *Littorina littorea* crawls up the vertical (inside) wall of a beaker in a dark aquarium, though the sea-water be better supplied with oxygen at the bottom than at the top. It is negatively geotropic.

2. This snail is negatively heliotropic.

3. The posterior region of the snail has a greater specific gravity than the anterior region.

4. In sea-water, the larger the angle of inclination (to the horizontal) of the surface on which the animals move the larger is the number of negatively geotropic animals; and the smaller the angle of inclination, the larger the number of animals which move downward and are perhaps positively geotropic.

5. In the air, the number of animals showing negative geotropism is always higher than that in the sea-water.

6. On a ground glass plate, the animals are less negatively geotropic than on a plain glass plate.

7. On a dry plain glass plate, a number of individuals oriented positively and crawled downward even at the angle of 90° (vertical) though this never happens on the moist plate. This is more striking on a dry wooden plate.

8. When the animals are placed with their heads down on a dry wooden plate, the highest percentage of positive geotropism is obtained.

9. The snails "hesitate" at the surface-film of sea-water, when light is not excluded.

10. In the direct sunlight, the snails are apparently more positively geotropic than in darkness, due to the fact that they are negatively heliotropic.

11. In the direct sunlight, 58 per cent. of the animals orient themselves head downward and crawl in that direction through the surface-film of sea-water into it.

12. In darkness, the snails, which "hesitated" at the surface-film of sea-water in the daylight, crawl upward *through* the film.

13. From the experimental results which the writer has obtained, he concludes that neither the mechanical theory, nor the pressure theory, nor the resistance theory is adequate to explain the phenomenon of the negative geotropism of *Littorina littorea* but a physiological one, that is, the statocyst or statolith theory. This theory is the more likely since these snails have statoliths (17, pp. 119–120). The writer, however, has no direct evidence, at present, in favor of the statolith theory. He is led to accept it largely by the method of exclusion. Furthermore evidence from many sides based on the experiments of the writer causes him to conclude that the surface-film theory is also not correct.

In conclusion, the writer wishes here to acknowledge his indebtedness to Professors Walter E. Garrey, Ralph S. Lillie, and Elias P. Lyon, for their valuable suggestions and criticism on his experiments at the Marine Biological Laboratory at Woods Hole, Mass., during the summers of 1912 and 1913. His thanks are also due to Professor Frank R. Lillie for the privileges of the Laboratory and to Professor Lyon for criticism and suggestions in the preparation of manuscript.

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# THE GEOTROPISM OF FRESHWATER SNAILS.[1]

SAKYO KANDA.

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## I. INTRODUCTORY.

Walter (10) and Dawson (2) have investigated the geotropic reactions of *Physa* and other freshwater snails in connection with their respiratory phenomena. However, their observations do not agree on certain points. Walter (10, p. 26) and Dawson (2, p. 93) agree with each other that freshwater snails are negatively geotropic, "when their lungs are empty." The snails being air-breathing forms, it is, of course, necessary for them to crawl up to the surface of water for their air supply, "although their specific gravity is meanwhile gradually increasing through exhaustion of the air," as Walter expresses it. For this upward crawling, the pull of gravity would be expected to act as a "directive force."

Walter and Dawson, however, depart from each other when they come to consider positive geotropism in these snails. The former states that, "after filling the lung with air, they are

[1] From the Physiological Laboratory of the University of Minnesota, Minneapolis.

positively geotropic," so that "they tend to climb down." The latter, on the other hand, states that when the snails "have sufficient air they become indifferent to gravity and crawl in all directions."

Moreover, they do not agree concerning the behavior of the snails from which "the air supply is cut off." According to Walter, "after reaching the highest point in the flask," which was in an inverted position in water, "and finding themselves unable to renew their supply, their ordinary behavior, to which there were some exceptions, was to let go and drop like dead weights" (10, p. 27). Dawson denies this statement of Walter as follows: "*Physa*, after they have been denied atmospheric air for some time, manifest indifference to the influence of gravity, and scatter over the sides and bottom of the bottle. They have never been observed to let go and drop like dead weights upon being denied atmospheric air" (2, pp. 104, 105).

In this paper an attempt has been made to compare certain experimental results obtained by the writer with those of his predecessors. A comparison is also made with other results obtained by the writer with marine snails.

The experimental work was done in the physiological laboratory of the University of Minnesota, under the direction of Professor E. P. Lyon, during the academic year of 1913–1914, while the writer was holding a Shevlin Fellowship. The writer expresses his appreciation of the interest and suggestions of Professor Lyon throughout the course of the work. To Professor John M. Holzinger, the principal of the State Normal School of Winona, Minn., the writer acknowledges indebtedness for the identification of the forms experimented upon.

## II. Materials.

Common freshwater snails, *Physa gyrina* Say, *Planorbis trivolvis*, *Limnæa stagnalis*, and *Limnæa Columella*, were used for the work. The snails were kept in glass aquaria together with green algæ. They seemed to be perfectly healthy, and were observed to grow.

## III. Experimental.

To study the behavior of an animal it is always necessary to discriminate the force under consideration as much as possible from other forces which may act simultaneously with it, favorably or antagonistically. Oxygen for pulmonate animals such as *Physa* is, of course, very important. That food is another factor in determining behavior of living animals need hardly be mentioned. Contact stimuli must also be considered. Light is often important. These with gravity are the chief forces which should be borne in mind.

The effect of the force of gravity on *Physa* and others is the problem with which this paper is chiefly concerned. But in considering this the other forces just mentioned must also be considered. Light especially must be taken into account.

### 1. *Heliotropism of Physa gyrina Say.*

Walter (10, pp. 23–24) has experimentally shown that *Physa primeana* Tyron and others are generally negatively heliotropic. A series of experiments was made with *Physa gyrina* Say to compare results with the results obtained by Walter. The experiments were conducted as follows: Five selected individuals were placed on a smooth glass plate with their anterior ends facing direct sunlight. The surface of the plate was carefully moistened. During experiments the angle of the rays of sunlight was about 22.5°. The glass plate was horizontally placed in air.

### Table I.

Heliotropism of *Physa* on a Horizontal Moist Glass Plate in Air at the Angle of 22.5° of the Rays of Sunlight.

Table shows results after one minute. Feb. 14, 1914, 9:30 A. M. Temperature, 22° C.

| No. of Animals. | No. of Trials. | — Heliotropism. | | + Heliotropism. | | Horizontally Crawled. | |
|---|---|---|---|---|---|---|---|
| | | No. | %. | No. | %. | No. | %. |
| 1 | 20 | 20 | 100 | 0 | 0 | 0 | 0 |
| 2 | 20 | 20 | 100 | 0 | 0 | 0 | 0 |
| 3 | 20 | 20 | 100 | 0 | 0 | 0 | 0 |
| 4 | 20 | 10 | 50 | 4 | 20 | 6 | 30 |
| 5 | 20 | 4 | 20 | 16 | 80 | 0 | 0 |
| Total 5 | 100 | 74 | or 74% | 20 | or 20% | 6 | or 6% |

The results, given in Table I. confirm Walter's conclusion. However, there are marked individual differences. Number 5, for instance, was exceptionally positive to light, while Numbers 1, 2, and 3 were always negative. Nevertheless, 74 per cent. were negative to light, and only 20 per cent. positive. From these results, the conclusion may be drawn that *Physa gyrina* Say is generally negatively heliotropic.

Dawson (2, pp. 60–61), on the other hand, has observed that darkness interferes with the activity of *Physa*. The writer's observations seem to be in accord with Dawson's. Five of the individuals above mentioned were kept in water under frequent observation one afternoon (from 2.40–4 P. M.) in darkness. Three of them were observed to crawl to the surface and then down again only two or three times; and two of them only once during the period, one 55 minutes and the other 70 minutes after being covered. The rest of the time they did not move at all.

### 2. *Geotropism of Physa and Other Species, with the Lung Empty and with the Lung Filled with Air.*

As already mentioned, Walter and Dawson disagree on their observations concerning positive geotropism in *Physa* and other species after the air supply is cut off. Neither has, however, furnished the quantitative evidence from which the conclusion was drawn. The writer, therefore, has made some quantitative observations on this point.

(*a*) *Observations on Physa.*—Five selected individuals of *Physa* were placed in a beaker containing about 300 c.c. of water. The beaker, which had vertical sides and a horizontal bottom, was placed as near as possible in optimum daylight. The water which was used for this purpose was taken from the dish in which *Physa* were kept, and was filtered when necessary. The following observations have two aspects, (1) response of *Physa* to gravity, when the lung is empty, and (2) when the lung is full of air. The results are given in Table II.

The negative geotropism of *Physa*, when its lung is empty, is precise and marked. It is impossible to mistake it. It is also evident, on the other hand, that the positive geotropism of the animal after filling the lung, though not as precise as the negative

geotropism observed when the lung is empty, is predominant in the majority of cases, that is, in over 90 per cent. Cases of "indifference" to gravity numbered less than 10 per cent. That *Physa* should become positive to gravity after taking in air supply seems to the writer to be quite natural, since the animal is primarily positively geotropic as will be shown later. Moreover, it is worth mention that each individual occasionally showed a peculiar "habit." It crawled up as usual, turned downward when it had reached the surface, and crawled down, making no effort to get air. The number of observations of this phenomenon for each individual is given for reference in Table II. in the second horizontal column and indicated by a star.

TABLE II.

GEOTROPISM OF *Physa* AT THE ANGLE OF 90° OF INCLINATION OF A SUPPORT IN WATER, AFTER ANIMALS HAVE TAKEN AIR IN THE "LUNG-SAC."

| Dates. | Temp. of Water. | No. of Trials. | Animal No. | + Geotropism. | | "Indifferent to Gravity" (?) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Vertically or almost Vertically Oriented Downward and Crawled. | Diagonally Oriented Downward and Crawled. | Horizontally Oriented and Crawled. | Irregularly Crawled. | Crawled on the Surface-Film of Water. |
| Dec. 27, 3.10–4.05 P.M.<br>Dec. 28, 3.25–3.50 P.M.<br>Dec. 31, 10.35–11.15 A.M. | 19° C. | 18 | 1 | 7 | 3 | 2 | 1 | 1 |
| | | | 1* | 3 | 1 | 0 | 0 | 0 |
| | | 15 | 2 | 6 | 5 | 0 | 0 | 1 |
| | | | 2* | 3 | 0 | 0 | 0 | 0 |
| | | 11 | 3 | 5 | 1 | 0 | 1 | 0 |
| | | | 3* | 4 | 0 | 0 | 0 | 0 |
| | | 11 | 4 | 8 | 0 | 0 | 0 | 0 |
| | | | 4* | 1 | 2 | 0 | 0 | 0 |
| | | 19 | 5 | 13 | 0 | 0 | 0 | 1 |
| | | | 5* | 5 | 0 | 0 | 0 | 0 |
| | | 74 | 5 | 67 or 90.5% | | 7 or 9.4% | | |

(*b*) *Observations on Planorbis and Limnæa.*—With single individuals of these genera, the same tests as above were made and still more conclusive results were obtained. Since these snails were comparatively large forms, they were favorable for observation. If they were dislodged they fell to the bottom, provided their lungs were "relatively empty." If the lungs on the contrary were full of air, dislodged snails floated on the surface

of water. The same was true in the case of *Physa*. But with this species, as has been already pointed out, it was impossible to exclude light in the experiments because it was inactive in darkness. Consequently the negative heliotropism tended to blur the geotropism. This disadvantage was entirely removed in *Planorbis* and *Limnæa*, because these forms were active even in total darkness.

Observation 1: *Planorbis trivolvis* was put in the 600-c.c. beaker full of water. It either floated or sank, depending on the condition of its lung as above mentioned. If it floated, *i. e.*, was lighter than water, observation on positive geotropism was made; if it sank, *i. e.*, was heavier than water, observation on negative geotropism was made. Either event, therefore, was useful. At about two-minute intervals, the snail was dislodged by a test-tube cleaner, and then was covered by a dark-box. The results are given in Table III.

TABLE III.

| Geotropism of *Planorbis trivolvis* on a Vertical Side of a Beaker Full of Water in Total Darkness When it Was Lighter than the Water. Table Shows Results Within Two Minutes. | | | | Geotropism of *Planorbis trivolvis* on a Vertical Side of a Beaker Full of Water in Total Darkness When it Was Heavier than the Water. Table Shows Results After Two Minutes. | | | |
|---|---|---|---|---|---|---|---|
| Temp. of Water. | No. of Trials. | + Geotropism. | | Temp. of Water. | No. of Trials. | — Geotropism. | |
| | | No. | %. | | | No. | %. |
| 19° C. | 30 | 30 | 100 | 19° C. | 10 | 10 | 100 |

Discussion is hardly needed. A hundred per cent. in both positive and negative geotropisms was obtained. The orientation of positive geotropism in this snail was just as precise as that of negative geotropism.

Observation 2: In the same manner as above, *Limnæa stagnalis* was observed in total darkness. The results are given in Table IV.

TABLE IV.

| Geotropism of *Limnæa stagnalis* on a Vertical Side of a Beaker of Water in Total Darkness When it Was Lighter than Water. Table Shows Results After Two Minutes. | | | | | Geotropism of *Limnæa stagnalis* on a Vertical Side of a Beaker of Water in Total Darkness When it Was Heavier than Water. Table Shows Results After Two Minutes. | | | |
|---|---|---|---|---|---|---|---|---|
| Temp. of Water. | No. of Trials. | + Geotropism. | | Did Not Move. | Temp. of Water. | No. of Trials. | — Geotropism. | |
| | | No. | %. | | | | No. | %. |
| 19° C. | 35 | 24 | 68.5 | 11 or 31.4% | 19° C. | 25 | 25 | 100 |

As may be seen from the table, the snail did not respond eleven times out of thirty-five trials, when it was lighter than water. This means that it was still floating when the complete two-minute interval was over. The failure of response to gravity in these cases need not be interpreted as "indifference" to gravity, because it was often observed that the snail had opened its air cavity at the time of observation. Evidently it was taking in more air. The geotropic response failed, therefore, because the lung was not full of air. All internal conditions being equal, the snail tends to crawl down, if its lung is full of air. If it crawled downward, it crawled vertically, orienting itself with its anterior end accurately in that direction.

Observation 3: *Limnæa columella*, again, when lighter than water, failed to respond to gravity nearly half the time, as Table V. has shown.

TABLE V.

Geotropism of *Limnæa columella* on a Vertical Side of a Beaker Full of Water in Total Darkness, When it was *Lighter* than the Water. Table Shows Results After Two Minutes.

| Temp. of Water. | No. of Trials. | + Geotropism. | | Did Not Move. |
|---|---|---|---|---|
| | | No. | % | |
| 19° C | 35 | 18 | 51.4 | 17 or 48.5% |

Only a limited number of observations could be made at a sitting, as the animals ceased to respond at all. This was probably a fatigue effect.

It was observed three or four times that the snail crawled down with its shell in a horizontal position; and two or three times with the anterior end of its shell pointed up. It thus crawled down even against mechanical disadvantage.

The writer was unable to obtain satisfactory observations on this snail's negative geotropism. It crawled up to the surface for air. But when it was subjected to experimentation, it retreated into its shell and did not readily come out.

The writer by the above observations confirms Walter's conclusion regarding the effect of taking air on the geotropism of *Physa* and other species.

### 3. *Geotropism of Physa with Lung Empty and Filled with Air, in Presence of Food.*

Food being one of the strongest of forces in determining behavior, it was thought advisable to observe its effect on *Physa*. Green algæ were carefully placed on the bottom of the beaker in which there were five selected individuals of *Physa*. It was surprising to find that with food present *Physa* seldom crawled up to the surface for the air supply. After obtaining air moreover most of them crawled down just as precisely as they crawled up. It must also be added that they were not in a starving condition previous to these experiments.

It should be remembered, however, that in this case three forces, that is to say, (1) light, to which *Physa* is negative, (2) gravity, to which it is positive after taking in air, and (3) food, to which it is presumably positive, were here combined. The result of the combination of these three forces is the acceleration of positive geotropism. Negative geotropism, on the other hand, is retarded, even though it is very important for the air supply.

### 4. *Geotropism of Physa at the Different Angles of Inclination of the Supports in the Air and in Total Darkness.*

Imagining that negative and positive geotropisms of *Physa* and others are due only to respiratory phenomena, Walter claims that "so far as gravity alone is concerned, they should show no response at all" (10, p. 26). According to Dawson, geotropism (negative) is only possible "when their lungs are empty, but when they have sufficient air they become indifferent to gravity and crawl in all directions" (2, p. 93). In a certain sense, therefore, Walter and Dawson agree on this point. This contention will be experimentally examined in this section.

(*a*) *Experiments with a Plain Glass Plate.*—After a few trials it was found that *Physa* was *positively geotropic* even at a slight inclination of a plain glass plate in the air and in total darkness. The following method of experimentation was therefore adopted. The well-moistened glass plate being held slightly inclined, the animal was placed upon it with its head down. When five selected individuals had been so placed, the plate was reversed,

and was placed on a "rack" specially made for angle determination. The whole arrangement was covered as soon as possible with a dark box. Curiously enough, darkness seemed not to interfere very much with *Physa's* activity, if the experiment was conducted in the air, though it did interfere in water, as already shown. *Physa* oriented itself in the line of the force of gravity and crawled in that direction. The relative weight of *Physa* is about a thousand times as great in the air as in the water. This probably made a difference in its activity in the air even in total darkness.

The results given in Table VI. show that from the angle of 10¾° of inclination to that of 56¼°, positive geotropism increases as the degree of the angle increases. It should be added that this was not because the lung was full of air. On the contrary, about two thirds of these *positive* animals sank, when they were tested in water. This means that their lungs were empty. Negative geotropism and horizontal crawling, on the other hand, decrease in reverse proportion as the angle of inclination is increased. This was not because the lung was empty. On the contrary, about half of these *negative* animals floated, when they were tested in water.

But there was a limit to the degree of inclination of the support, beyond which *Physa* could not actively move on the plain glass plate on account of the force of gravity. This is significant. At the angle of 67½° positive geotropism suddenly decreases, and negative geotropism and horizontal crawling increase. Gravity, of course, is constant and always exerted vertically. But the effective force exerted on the animals depends upon the inclination of the surface on which the animals crawl. The exertion required to enable the animals to move on a horizontal surface is least; that required on a vertical surface is greatest. At the angle of 67½°, therefore, the effective force of gravity was so great that some individuals of *Physa* could not actively move against it (in air). An apparent increase of negative geotropism, therefore, was the result. This becomes clear, when one considers the failure of the experiments at the angle of 78¾°, which was due to the fact that nearly all the animals passively slid down the plate, mostly with their heads up. The optimum inclination

seems to be about 56°. The inference may be made that positive geotropism in *Physa* is an active process, and that negative geotropism, on the other hand,is due largely, though not entirely, to "passive orientation."

The so called mechanical theory of geotropism, however, can not be applied even in the case of negative geotropism. This is obvious when one remembers that *Physa* becomes negative to gravity when "its lung is empty," even though its specific gravity is less than that of water; and positive when its lung is full of air, even though its specific gravity is greater than that of water. The essential factors, therefore, which determine the geotropic orientation, either positive or negative, of *Physa* seem to be internal, that is, physiological ones (cf. 3, 4, 5, 7, 8 and 9 in the bibliography).

## TABLE VI.

GEOTROPISM OF *Physa* AT THE DIFFERENT ANGLES OF INCLINATION OF A SMOOTH GLASS PLATE IN THE AIR IN TOTAL DARKNESS.

At beginning of experiments, each head placed upward. Table shows results after one minute.

| Temp. of Room. | Angles. | No. of Trials. | No. of Animals. | + Geotropism. Oriented and Crawled Downward. | | − Geotropism. Crawled Upward. | | Horizontally Crawled. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % |
| 20.5° C | 67.5° | 5 | 50 | 29 | 58 | 11 | 22 | 10 | 20 |
| 20.5° C | 56¼° | 5 | 50 | 47 | 94 | 1 | 2 | 2 | 4 |
| 20.5° C | 45° | 5 | 50 | 41 | 82 | 6 | 12 | 3 | 6 |
| 20.5° C | 33¼° | 5 | 50 | 35 | 70 | 11 | 22 | 4 | 8 |
| 20.5° C | 22½° | 5 | 50 | 31 | 62 | 12 | 24 | 7 | 14 |
| 20.5° C | 11¼° | 5 | 50 | 30 | 60 | 11 | 22 | 9 | 18 |
| Total ..... | | 30 | 300 | 213 or 71% | | 52 or 19.3% | | 35 or 11.6% | |

From the above results the writer thinks that both Walter and Dawson overlooked the fact that *Physa* is naturally positively geotropic.

(*b*) *Experiments with a Ground-Glass Plate.*—Contact stimuli, as stated, affect the behavior of animals. Supposedly it might affect the geotropism of *Physa*. The same methods were used here as in the above. The results given in Table VII. show a fair agreement with the above supposition.

## TABLE VII.

GEOTROPISM OF *Physa* AT THE DIFFERENT ANGLES OF INCLINATION OF A GROUND GLASS PLATE IN AIR IN TOTAL DARKNESS.

At beginning of experiment each head placed upward. Table shows results after one minute.

| Temp. of Room. | Angles. | No. of Trials. | No. of Animals. | +Geotropism. Oriented and Crawled Down. | | −Geotropism. Crawled Up Not Quite Vertical. | | Horizontally Crawled. | | Not Moved. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % |
| 20° C. | 90° | 10 | 50 | 38 | 76 | 0 | 0 | 5 | 10 | 7 | 14 |
| | 78¾° | 10 | 50 | 41 | 82 | 1 | 2 | 5 | 10 | 3 | 6 |
| | 67½° | 10 | 50 | 41 | 82 | 3 | 6 | 1 | 2 | 5 | 10 |
| | 56¼° | 10 | 50 | 37 | 74 | 5 | 10 | 2 | 4 | 6 | 12 |
| | 45° | 5 | 50 | 35 | 70 | 2 | 4 | 5 | 10 | 8 | 16 |
| | 33¼° | 5 | 50 | 32 | 64 | 3 | 6 | 8 | 16 | 7 | 14 |
| | 22½° | 5 | 50 | 28 | 56 | 5 | 10 | 4 | 8 | 13 | 26 |
| | 11¼° | 5 | 50 | 24 | 48 | 11 | 22 | 11 | 22 | 4 | 8 |
| Total..... | | 60 | 400 | 276 or 69% | | 30 or 7.5% | | 41 or 10% | | 53 or 13.5% | |

*Physa* evidently could stick on the ground-glass plate better than on the plain glass plate, as would be expected. Experiments, therefore, could be carried on even at an angle of 90°. Here again, it is noticeable that there is a decrease of positive geotropism at this angle. The optimum inclination in this case is between the angles of 67½° and 78¾°.

### 5. *Summation of Gravity and Light Stimuli.*

*Physa* being positive to gravity and negative to light, as already seen, it would be expected to crawl downward even at a small angle of inclination, if it were placed in a strong light. This is just what happened. One morning the rays of sunlight were falling at an angle of about 11¾°. The angle of the rays of sunlight was nearly constant during the experiments. Ten selected individuals were carefully placed with their heads down on a moist plain glass plate. The plate was then reversed and put on the rack whose angle of inclination was 11¾°. They all oriented themselves away from the rays of sunlight, that is, downward, and crawled in that direction. Ten trials were made and there was no exception.

Besides the above, observations on exclusion of the air were attempted, but the results were not satisfactory. Generally speaking however, Dawson's observations seem to be right, although the writer observed one individual "drop" once.

## IV. Summary and Conclusion.

1. *Physa gyrina* Say is negatively heliotropic. It becomes sluggish in its activity in darkness, particularly in water.

2. *Physa gyrina* Say, *Planorbis trivolvis*, *Limnæa stagnalis*, and *Limnæa columella*, are negatively geotropic when their lungs are empty; and positively geotropic when their lungs are full of air. *Physa* often comes near the surface and crawls down again without filling its lung with air.

3. *Physa*, when put with green algæ, does not often crawl to the top.

4. At different angles of inclination of a plain glass plate in the air and in total darkness, *Physa* is positively geotropic. There is a certain limit of inclination beyond which the animal can not actively move on account of the force of gravity.

(*a*) Many of the individuals in question are positive to gravity, even though their lungs are empty.

(*b*) The optimum inclination of the plain glass plate on which *Physa* may crawl is an angle of 56¼°.

(*c*) At the angle of 67½°, positive geotropism decreases as negative geotropism and horizontal crawling increase. The negative geotropism is not necessarily the result of lack of oxygen.

(*d*) At the angle of 78¾° no experiments are successful.

5. At different angles of inclination of a ground-glass plate in the air and in total darkness, *Physa* reacts to gravity in a similar manner as though on the plain glass plate, although the limit of inclination is a little higher in the former than in the latter. The optimum inclination of the ground-glass plate is between the angles of 67½° and 78¾°.

6. Contact stimuli seem to interfere slightly with the geotropism of *Physa*.

7. The combination of gravity and light (both the glass support and the rays of light being inclined 11¾° to the horizontal) accelerates positive geotropism of *Physa*.

From the data here given the writer is inclined to draw the conclusion that *Physa* is naturally positively geotropic. It is little wonder, therefore, that *Physa* becomes positive to gravity when its lung is filled with air.

As to the organ of geotropic orientation of *Physa* and other snails, no direct experimental evidence is yet furnished. But

according to Cooke (1, p. 196), *Limnæa*, *Planorbis* and *Physa* have statocysts, which are situated near the pedal ganglion, and are probably connected with the cerebral. The statocyst also contains statoliths. The number of the statoliths "varies in different genera and species." There are a hundred in *Limnæa stagnalis* on which the writer has experimented, but about fifty in *Planorbis contratus* and *Physa fontinalis*. Therefore, *Planorbis trivolvis* and *Physa gyrina* Say very probably have statoliths. These statocysts with statoliths may be the organs for geotropic orientation. At any rate, the most probable factors of geotropic orientation, positive or negative, seem to be internal, that is, physiological, and not external.

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